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RESEARCH INSTITUTE, NEW DELHI**

**I.A.R.I.6.**

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# JOURNAL OF DAIRY SCIENCE

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# WHAT IS Duraglas?

TRADE-MARK

REG. U. S. PAT. OFF.

YOU have seen our Duraglas advertisements in national magazines.\* Now let us explain Duraglas more fully, point out its advantages to you in this industry.

Duraglas is not an advertising catchword casually adopted for our containers. The mere addition of a name to containers adds nothing to their quality.

NO—Duraglas is far more than that. Duraglas is the trade-mark that symbolizes the fruition of a glassmaking technique developed by scientific research. Only after our technique reached the perfection we set for ourselves, did we give it the name Duraglas.

## *A Step Forward for an Age-Old Industry*

Duraglas is no overnight discovery. There is no "secret ingredient." But the Duraglas method—a milepost in the technological progress of an industry 5,000 years old—does represent epoch-making advances in every step of producing glass containers. Duraglas cannot be duplicated without painstaking research, special equipment and the experience which comes from making more glass containers, year after year, than any other manufacturer.

## *Duraglas Means a Better Container*

To containers made by this technique, we give the name "Duraglas." They have greater

strength and durability. In the case of returnable containers—milk, beverage and beer bottles—Duraglas results in longer life and lower trip costs. In one-trip containers, the strength of Duraglas makes possible lower weight, with resulting important economies. Where safe delivery of contents is paramount, Duraglas bottles offer greater confidence.

Duraglas brings better packaging not only to industries that normally use glass, but to hundreds of others whose products would serve and sell better in glass containers.

## *Duraglas is to Glass What Sterling is to Silver*

We have established for Duraglas a standard to be zealously guarded.

Although most of the containers we now make are Duraglas, some few are not. Good containers though they are—the equal certainly of others in their fields—they are not yet called Duraglas. For each container group presents special problems; and month by month, as the Duraglas method is fitted to these containers, they will be awarded the trade-mark "Duraglas."

The Duraglas standard is fixed, not by law as is sterling, but by our experience and integrity... gives new meaning to our slogan—"First in Glass."

Owens-Illinois Glass Company, Toledo, Ohio.

*\*In The Saturday Evening Post, Collier's and Life*



# OWENS-ILLINOIS GLASS

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Vol. XXV, No. 2, February,

# Pioneering of the Owens-Illinois Handi-Quart is Divided Into 3 Parts

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Milk, all liquid—even molten glass—when poured forms a "tear-drop." That's Nature's way of evenly distributing liquid mass, of insuring best distribution of thickness and weight. The Handi-Quart is a natural "tear-drop"—modified to traditional milk bottle shape.

## PART 3... Resultant Economies of Handi-Quart

Bear in mind that these amazing economies—extended to the approximately 12 billion glass containers of milk delivered yearly in America—would save 1,595,000 tons weight alone. More, since 100 Handi-Quarts can be made from the same amount of glass needed for 76 old-style quarts, much glass can be released for defense!

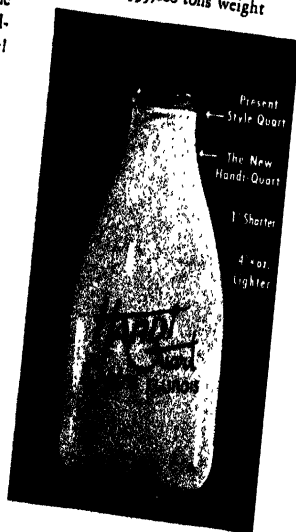
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**FOR CONSUMERS**... The O-I Handi-Quart is easier to hold and pour from—easier to slide into refrigerator shelves.

We are proud indeed of our part in pioneering the development of this distinctly economical and perfect container for milk.

**OWENS-ILLINOIS** Complete Dairy Service  
TOLSON, OHIO

WHERE QUALITY PREDOMINATES



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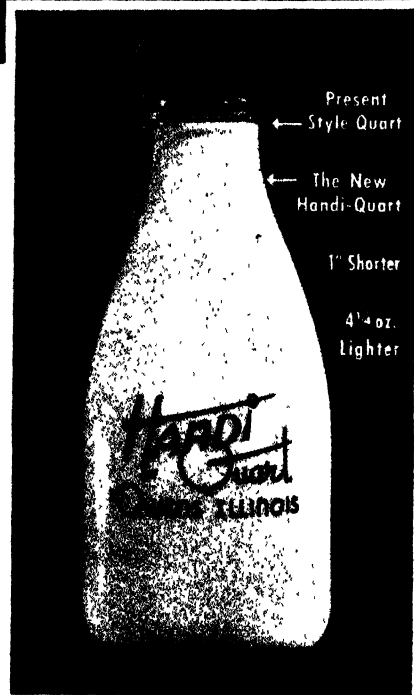
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# Pioneered by Owens-Illinois, the Handi-Quart fits today's need for Conservation

Each year, Dairymen deliver around 12 billion glass containers of milk. Thus the 4¼-oz. weight saving of each O-I Handi-Quart can mean . . . over 1½ million tons *less* weight to be lifted, trucked and paid for. More, since 100 Handi-Quarts can be made from the same amount of glass needed for 76 old-style quarts, much glass can be released for defense!

## Five Tangible Savings

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## Applied Color Lettering Adds Merchandising Distinction

Handi-Quarts provide an extremely low-cost multi-impression merchandise medium. This, through Applied Color Lettering, can be used . . . 1. To build good will and prestige. 2. To introduce and boost sales of other milk products. 3. To keep your name "distinctively" before customers and prospects.

Made by the Duraglas technique, all O-I bottles are of uniform capacity, color and luster, since all phases of their quality are *scientifically controlled*.



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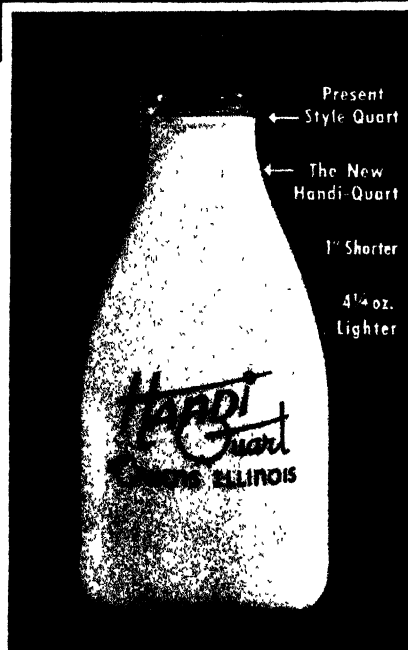
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Vol. XXV, No. 6, June, 1942

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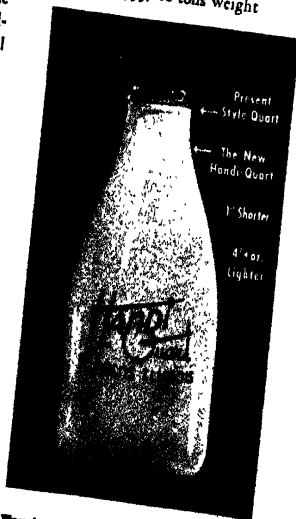
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# JOURNAL OF DAIRY SCIENCE

VOLUME XXV

JANUARY, 1942

NUMBER 1

## A COMPARISON OF HOT WATER, STEAM AND CHLORINE FOR SANITIZING ICE CREAM FREEZERS\*

F. W. FABIAN, A. E. HOOK, AND G. L. NIELSEN

*Michigan Agricultural Experiment Station, East Lansing, Michigan*

*Cooperating with G. J. TURNEY, Chief Milk Inspector,  
Lansing Department of Health, Lansing, Michigan*

The ice cream freezer is an important factor in the production of low bacterial count ice cream since the introduction of better and more sanitary methods for the preparation of ice cream mix containing relatively few bacteria. The present work was done in order to study the relative efficiency of the most commonly used physical and chemical methods

### METHOD

In the experiments reported herein, three ice cream plants were chosen. one was a small counterfreezer installation and two were commercial plants. The type of freezer in use in Plant A was a Mills 20-quart horizontal counterfreezer. Plant B used a 50-quart Creamery Package Mfg. Company batch freezer and Plant C a 40-quart Miller batch freezer.

The method of cleaning all freezers preparatory to sanitizing them was standardized. This was done so that the cleaning operations would be the same throughout and the bacterial reduction resulting from the cleaning of the freezers would be as nearly uniform as possible.

The same ice cream mix was used for testing the efficiency of each type of sanitization so that there would be approximately the same numbers and types of bacteria present to be killed. The ice cream mix used was a commercial one prepared in the usual way and allowed to stand for two days at room temperature until sufficient bacterial growth had taken place so as to insure the presence of sufficient numbers of bacteria in the freezer after it had been cleaned ready for sanitization.

### *Method of Cleaning Freezers.*

1. Three, six and nine gallons respectively of cold water (60° F.) were placed in the freezer and the freezer run for two minutes.

2. Next, three, six and nine gallons respectively of warm water (140–

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\* Journal Article No. 543 (n.s.).

150° F.) to which had been added 0.5 per cent of Wyandotte dairy cleaner were placed in the freezer and the freezer run for two minutes.

3. Finally, three, six and nine gallons respectively of hot water (180° F.) were placed in the freezer and the freezer run for two minutes.

All the freezers were first cleaned in the above manner irrespective of the method which was to be used later in sanitizing them.

#### *Hot Water Sanitization.*

In the hot water method of sanitizing three, six and nine gallons, respectively, of water at approximately 200° F. were added to the freezer after it had been cleaned by the method previously outlined. The hot water was agitated in the freezer by running it for two minutes. After the last water had been drained from the freezer, one liter of sterile water was placed in the machine and agitated by running the freezer. (Tables 1, 4, 5 and 6.)

Samples of the mix and all the various rinse waters were taken for bacterial analysis. After the freezer was sanitized, it was disassembled and swabs made of the front and rear bearings. In one type of freezer, it was impossible to get a swab of the rear bearing due to the improper operation of the freezer over a long period. All samples were plated immediately on Standard agar for total plate count and on violet-red-bile agar for *Escherichia coli* counts. These data are given in table 4.

The temperature of the rinse waters was taken as they entered and left the freezer. In collecting these data, the freezer was operated according to the method in use in the factory where the ice cream was made. The results are given in tables 1, 4, 5, and 6.

#### *Steam Sanitization.*

After the freezer had been cleaned by the method just outlined, the steam hose was placed in the freezer and the steam turned on and allowed to run until the condensed steam dripping from the freezer had a temperature of 190° F.

At the end of this period the freezer was rinsed with one liter of sterile water and the water plated for bacterial counts. The head was removed and the bearings swabbed. The results are given in tables 2, 4, 5, and 6.

#### *Chlorine Sanitization.*

Chlorine or chemical sanitization was carried out the same as in the two types of physical sanitization except the freezers were rinsed with three gallons of cold water containing 94.5 to 132.65 p.p.m. of active chlorine after the freezers had been cleaned.

The freezers were then rinsed with one liter of sterile water which was collected in a flask containing sufficient sodium thiosulphate to neutralize the effect of the chlorine. Swabs were made of the front and rear bearings. The complete data are in tables 3, 4, 5 and 6.

*Bacteriological Analysis.*

Platings were made in duplicate from all rinse waters on Standard milk agar. They were incubated at 37° C. for 48 hours and counted. All procedures were according to Standard Methods for the Examination of Dairy Products (2).

The front and rear bearings where possible were swabbed with sterile cotton swabs kept in tubes containing 10 ml. of sterile saline. The recommended procedure (4) for swabbing dishes and utensils was followed.

TABLE 1

*Temperatures of three gallons of water used to rinse freezers as it entered and as it left the freezers after a two minute rinse period during hot water sanitization*

Plant	Cold water 60° F. E-L	Hot water 140° F. E-L	Hot water 180° F. E-L	Hot water 200° F. E-L
	°F.	°F.	°F.	°F.
A	68-52	140-108	180-153	198-166
B				
Run # 1	60-40	144-10	184-46	207-63
B				
Run # 2	60-49	141-66	182-58	202-77
B				
Run # 3	57-38	146-52	184-86	198-120
C				
Run # 1	61-31	143-52	184-102	202-132
C				
Run # 2	59-36	142-56	180-96	201-124

E = temperature of the rinse water as it entered the freezer.

L = temperature of the rinse water as it left the freezer after a two minute rinse period.

The coliform count was determined by using violet-red-bile agar according to Standard Methods for the Examination of Dairy Products (2).

*Influence of Volume of Water Used to Rinse Freezers.*

It early became apparent from the data collected in tables 1, 2 and 3 that there was considerable variation in the temperature of the water as it left the freezers. In the first series of tests only three gallons of water were used for the various rinse waters. A second series of tests were set up using one of the freezers, a 50-quart Creamery Package Mfg. Co. batch freezer in Plant B in which the amount of rinse water was increased from three (tables 1, 2 and 3) to six and finally nine gallons.

*Proper Handling of Freezers.*

Another factor influencing the temperature of the rinse water is the handling of the freezer during the rinsing operation. To study the influence of these factors, one experiment was conducted as follows:



TABLE 2

*Temperature of three gallons of rinse water used to rinse freezers as it entered and as it left freezers after a two minute rinse period and temperature of steam drip after two minutes steaming*

Plant	Cold water 60° F. E-L	Hot water 140° F. E-L	Hot water 180° F. E-L	Temp. of steam drip
	°F.	°F.	°F.	
A	68-52	140-108	180-153	198-166° F* 3 gallons 2 minutes
B				
Run # 1	59-51	150-68	181-74	Steam to 197° F. drip
B				
Run # 2	61-75	140-103	176-127	Steam to 190° F. drip
B				
Run # 3	54-76	142-102	181-126	Steam to 191° F. drip
C				
Run # 1	58-43	141-82	177-117	Steam to 194° F. drip
C				
Run # 2	60-38	140-82	181-123	Steam to 190° F. drip

E = temperature of rinse water as it entered the freezer.

L = temperature of rinse water as it left the freezer.

\* = no steam available—hot water used.

TABLE 3

*Temperature of three gallons of water used to rinse freezers two minutes and parts per million of chlorine used in rinsing freezers for chlorine sanitization*

Plant	Cold water 60° F. E-L	Hot water 140° F. E-L	Hot water 180° F. E-L	Cold chlorine*
	°F.	°F.	°F.	
A	63-49	140-108	180-152	132.65-87.85 p.p.m. 2 minutes
B				
Run # 1	59-50	150-58	183-72	112.35-82.95 p.p.m. 2 minutes
B				
Run # 2	62-60	142-94	181-124	108.5-86.8 p.p.m. 2 minutes
B				
Run # 3	60-35	143-101	185-128	121.45-91.35 p.p.m. 2 minutes
C				
Run # 1	61-68	143-101	182-110	112.75-81.35 p.p.m. 2 minutes
C				
Run # 2	60-63	140-100	180-110	94.5-63.0 p.p.m. 2 minutes

E = temperature of the rinse water as it entered the freezer.

L = temperature of the rinse water as it left the freezer.

\* The first figure in this column represents the parts per million of chlorine at the beginning and the second figure the parts per million of chlorine at the end of the two minute rinse period.

TABLE 4

Showing the standard bacterial plate counts, percentage of organisms removed and the *Escherichia coli* counts of ice cream mix and the influence of each subsequent operation on the bacterial content of the freezers using three gallons of water for each rinsing of the freezer

Material plated out	Plant A—3 gallon rinse				Plant B—3-gallon rinse				Plant B—3-gallon rinse			
	Standard plate counts per ml.	Per cent organisms removed	<i>E. coli</i> counts per ml.	Standard plate counts per ml.	Per cent organisms removed	<i>E. coli</i> counts per ml.	Standard plate counts per ml.	Per cent organisms removed	<i>E. coli</i> counts per ml.	Standard plate counts per ml.	Per cent organisms removed	<i>E. coli</i> counts per ml.
<i>Hot Water Sanitization</i>												
1. Mix before starting	19,000,000	—	320,000	5,200,000	—	107	6,000,000	—	—	—	—	0
2. 60° rinse water	670,000	96.64	1,000	1,100,000	97.52	13	1,700,000	95.54	—	—	—	34
3. 140° rinse water	16,000	2.31	1,000	24,500	2.17	0	32,300	1.76	—	—	—	0
4. 180° rinse water	3,820	0.55	0	1,200	0.11	0	104,600	5.69	—	—	—	0
5. 200° rinse water	3,460	0.50	0	2,300	0.21	0	160	0.008	—	—	—	0
6. Sterile rinse water	2,640	—	0	97	—	0	65	—	—	—	—	0
7. Swab of front bearing	270*	—	10*	Not taken	—	—	250	—	—	—	—	0
8. Swab of rear bearing	510*	—	100*	Not taken	—	—	Not taken	—	—	—	—	—
<i>Steam Sanitization</i>												
1. Mix before starting	17,900,000	—	290,000	7,000,000	—	122	100,000	—	—	—	—	0
2. 60° rinse water	117,000	98.73	0	655,000	98.92	38	70,000	98.26	—	—	—	2
3. 140° rinse water	1,200	1.01	0	6,350	0.96	0	950	1.33	—	—	—	0
4. 180° rinse water	130	0.11	0	765	0.12	1	230	0.32	—	—	—	0
5. Sterile rinse water	171	0.14	0	30	0.005	0	60	0.085	—	—	—	0
6. Swab of front bearing	70*	—	0	Not taken	—	—	2,200	—	—	—	—	0
7. Swab of rear bearing	60*	—	0	Not taken	—	—	Not taken	—	—	—	—	—
<i>Chlorine Sanitization</i>												
1. Mix before starting	14,600,000	—	60,000	6,250,000	—	242	400,000	—	—	—	—	0
2. 60° rinse water	31,000	92.15	10,000	1,040,000	98.84	46	60,000	88.41	—	—	—	0
3. 140° rinse water	2,600	7.73	0	11,500	1.09	0	6,000	8.84	—	—	—	0
4. 180° rinse water	40	0.12	0	700	0.07	0	1,850	2.74	—	—	—	0
5. Chlorine rinse water	Not taken	—	—	13	0.003	0	12	0.002	—	—	—	0
6. Sterile rinse water	0	—	0	26	—	0	0	—	—	—	—	0
7. Swab of front bearing	20*	—	0	Not taken	—	—	Not taken	—	—	—	—	0
8. Swab of rear bearing	30*	—	0	Not taken	—	—	Not taken	—	—	—	—	—

\* All bacterial counts from the swabs are total counts.

TABLE 4—(Continued)

Material plated out	Plant C—3-gallon rinse				Plant C—3-gallon rinse				Plant C—3-gallon rinse			
	Standard plate counts per ml.	Per cent organisms removed	<i>E. coli</i> counts per ml.		Standard plate counts per ml.	Per cent organisms removed	<i>E. coli</i> counts per ml.		Standard plate counts per ml.	Per cent organisms removed	<i>E. coli</i> counts per ml.	
<i>Hot Water Sanitization</i>												
1. Mix before starting	760,000	.....	1,630		131,500,000	.....	10		456,000,000	.....	0	
2. 60° rinse water	200,000	25.284	106		29,300,000	99.43	37		18,410,000	97.06	3	
3. 140° rinse water	135,000	17.07	59		123,000	0.42	0		460,000	2.42	5	
4. 180° rinse water	381,000	48.17	4		29,000	0.10	1		51,900	0.27	7	
5. 200° rinse water	75,000	9.48	0		14,500	0.05	0		46,000	0.24	0	
6. Sterile rinse water	19,200	.....	0		4,100	.....	1		530	.....	00	
7. Swab of front bearing	8,910*	.....	50		36,750	.....	10		7,500	.....	0	
8. Swab of rear bearing	220,000	.....	0		765,000	.....	210		119,000	.....	200	
<i>Steam Sanitization</i>												
1. Mix before starting	450,000	.....	460		30,000,000	.....	0		353,000,000	.....	0	
2. 60° rinse water	130,000	59.04	145		1,705,000	97.54	8		1,890,000	80.32	1	
3. 140° rinse water	86,000	39.05	2		43,000	2.46	0		339,000	14.41	0	
4. 180° rinse water	3,000	1.36	2		5 pr.	.....	0		124,000	5.27	1	
5. Sterile rinse water	1,200	0.54	2		60	0.001	0		40	0.001	1	
6. Swab of front bearing	10	.....	20		1,800	.....	0		150	.....	0	
7. Swab of rear bearing	28,500	.....	80		27,900	.....	50		34,900	.....	0	
<i>Chlorine Sanitization</i>												
1. Mix before starting	1,300,000	.....	790		15,500,000	.....	0		311,000,000	.....	0	
2. 60° rinse water	415,000	77.58	112		6,600,000	98.10	41		58,300,000	97.95	5	
3. 140° rinse water	114,000	21.31	0		120,000	1.78	2		1,190,000	2.00	1	
4. 180° rinse water	5,870	1.10	3		7,600	0.11	0		30,300	0.05	0	
5. Chlorine rinse water	33	0.007	0		37	0.001	0		36	0.001	0	
6. Sterile rinse water	16	.....	1		spr.	.....	00		61	.....	0	
7. Swab of front bearing	4,990*	.....	0		57,000*	.....	80*		320*	.....	0	
8. Swab of rear bearing	67,500*	.....	10		396,700*	.....	40*		470*	.....	0	

\* All bacterial counts from the swabs are total counts.

TABLE 5

*Showing the standard bacterial plate counts, percentage of organisms removed and the Escherichia coli counts of ice cream mix and the influence of each subsequent operation on the bacterial content of the freezers using six gallons of water for rinsing of the freezer*

Material plated out	Plant B—6-gallon rinse				Plant B—6-gallon rinse				Plant B—6-gallon rinse			
	Standard plate counts per ml.	Per cent organisms removed	E. coli counts per ml.		Standard plate counts per ml.	Per cent organisms removed	E. coli counts per ml.		Standard plate counts per ml.	Per cent organisms removed	E. coli counts per ml.	
<i>Hot Water Sanitization</i>												
1. Mix before starting	4,300,000	.....	5,000		5,000,000	.....	200,000		2,500,000	.....	5,000	
2. 60° rinse water	1,900,000	99.69	500		1,300,000	87.92	127,000		450,000	96.71	500	
3. 140° rinse water	5,000	0.28	200		178,000	12.04	4,200		10,000	2.15	52	
4. 180° rinse water	450	0.02	3		550	0.04	1		3,500	0.75	56	
5. 200° rinse water	45	0.004	0		85	0.007	3		1,800	0.39	28	
6. Sterile rinse water	12	.....	0		22	.....	0		983	.....	0	
7. Swab of front bearing	90*	.....	0		1,080	.....	20		15,000	.....	0	
8. Swab of rear bearing	1,428*	.....	0		1,750	.....	280		(est.)	.....	0	
<i>Steam Sanitization</i>												
1. Mix before starting	3,000,000	.....	1,000		445,000,000	.....	0		1,500,000	.....	10,000	
2. 60° rinse water	700,000	98.28	0		63,000,000	99.90	209,500		950,000	99.73	200	
3. 140° rinse water	12,000	1.08	0		60,000	0.09	8,100		1,500	0.16	55	
4. 180° rinse water	250	0.03	0		3,000	0.005	89		1,020	0.11	11	
5. Sterile rinse water	3	0.006	0		1	.....	0		3	0.001	0	
6. Swab of front bearing	10*	.....	0		2,740	.....	142		0	.....	0	
7. Swab of rear bearing	670*	.....	0		150	.....	0		10	.....	0	
<i>Chlorine Sanitization</i>												
1. Mix before starting	3,300,000	.....	5,000		13,000,000	.....	650,000		2,000,000	.....	0	
2. 60° rinse water	550,000	98.67	0		2,100,000	78.12	215,900		1,210,000	98.73	0	
3. 140° rinse water	7,000	1.25	0		580,000	21.58	11,900		15,000	1.22	2	
4. 180° rinse water	400	0.07	0		8,050	0.30	1		500	0.04	28	
5. Chlorine rinse water	8	0.004	0		1	.....	0		.....	.....	0	
6. Sterile rinse water	5	.....	0		0	.....	0		.....	.....	0	
7. Swab of front bearing	10*	.....	0		80*	.....	0		15,000*	.....	10*	
8. Swab of rear bearing	1,415*	.....	0		1,120*	.....	280*		15,000*	.....	10*	

\* All bacterial counts from the swabs are total counts.

TABLE 6

Showing the standard bacterial plate counts, percentage of organisms removed and the *Escherichia coli* counts of ice cream mix and the influence of each subsequent operation on the bacterial content of the freezers using nine gallons of water to rinse the freezer

Material plated out	Plant B—9-gallon rinse			Plant B—9-gallon rinse			Plant B—9-gallon rinse		
	Standard plate counts per ml.	Per cent organisms removed	<i>E. coli</i> counts per ml.	Standard plate counts per ml.	Per cent organisms removed	<i>E. coli</i> counts per ml.	Standard plate counts per ml.	Per cent organisms removed	<i>E. coli</i> counts per ml.
<i>Hot Water Sanitization</i>									
1. Mix before starting	52,000,000	98.54	100,000	9,000,000	72.66	2,500	14,000,000	98.87	70,000
2. 60° rinse water	44,000,000	1.43	2,000	7,200,000	27.25	4,000	4,400,000	1.01	1,500
3. 140° rinse water	640,000	0.01	1,000	2,700,000	0.09	0	45,000	0.11	0
4. 180° rinse water	27,960	0.02	0	9,200	0.006	0	5,000	0.002	0
5. 200° rinse water	8,255	0	0	508	0	0	20	0	0
6. Sterile rinse water	74,300	0	2	5,143	0	4	317	0	0
7. Swab of front bearing	11,000	0	0	7,250	0	20	1,890	0	0
8. Swab of rear bearing	0	0	0	2,400	0	0	1,500	0	0
<i>Steam Sanitization</i>									
1. Mix before starting	8,500,000	98.10	0	7,300,000	96.43	80,000	63,000,000	95.14	60,000
2. 60° rinse water	6,200,000	1.74	0	5,800,000	3.46	8,500	7,100,000	4.82	9,500
3. 140° rinse water	110,000	0.08	0	208,000	0.11	0	360,000	0.03	0
4. 180° rinse water	5,000	0.08	0	6,000	0.003	0	2,400	0.001	0
5. Sterile rinse water	5,144	0	0	87	0	0	32	0	0
6. Swab of front bearing	9,530	0	0	350	0	0	1,480	0	0
7. Swab of rear bearing	5,080	0	0	700	0	0	510	0	0
<i>Chlorine Sanitization</i>									
1. Mix before starting	7,000,000	99.36	450,000	8,600,000	77.37	300,000	8,500,000	97.28	70,000
2. 60° rinse water	4,600,000	0.54	7,000	6,500,000	22.61	10,000	8,000,000	2.67	11,000
3. 140° rinse water	24,000	0.001	0	1,900,000	0.02	100	220,000	0.040	0
4. 180° rinse water	800	0.11	0	1,600	0.001	0	32,000	0	0
5. Chlorine rinse water	5,000	0	0	3	0	0	1	0	0
6. Sterile rinse water	1,715	0	0	3	0	0	1	0	0
7. Swab of front bearing	6,350*	0	0	6,350*	0	0	205*	0	0
8. Swab of rear bearing	10,000*	0	0	5,080*	0	0	2,500*	0	10*

\* All bacterial counts from the swabs are total counts.

The 50-quart freezer in Plant B, in a dry state, was allowed to come to  $-10^{\circ}\text{F}$ . The valve to the expansion chamber was then closed, but the liquid ammonia line was left open. Three gallons of  $60^{\circ}\text{F}$ . rinse water was poured into the freezer, and the freezer run for two minutes. The rinse water was then run off, and the temperature taken. This procedure was repeated using 6 and 9 gallons of rinse water. Results are shown in table 7.

A second experiment was performed with both the valve to the expansion chamber and the valve to the liquor line closed, so that the freezer was entirely shut off. The amounts of rinse water and the initial temperature of the rinse waters were the same as for the first experiment. The results are shown in table 8.

TABLE 7

*The effect of the amount of rinse water used to rinse an ice cream freezer on the temperature of the rinse water (expansion chamber closed; liquor line open)*

Trial No.	Initial temp. of rinse water	Temperature of rinse water after running freezer for 2 minutes		
		3-gallon rinse	6-gallon rinse	9-gallon rinse
	$^{\circ}\text{F}$ .	$^{\circ}\text{F}$ .	$^{\circ}\text{F}$ .	$^{\circ}\text{F}$ .
1	60	33	33	38
	140	40	51	65
	180	50	65	81
	200	53	71	88
2	60	34	33	35
	140	44	56	67
	180	49	71	82
	200	54	71	87
3	60	34	34	34
	140	44	53	66
	180	50	66	82
	200	55	67	88
4	60	33	33	34
	140	42	51	67
	180	48	67	81
	200	53	68	88
Average of four runs	60	33.5	33	35
	140	42.5	53	66
	180	49	67	81.5
	200	53	69	88

It is obvious from tables 7 and 8 that the amount of water used to rinse the freezer as well as the method of handling the freezer during rinsing has an influence on the temperature of the rinse water. This in turn would influence the sanitizing value of any of the three methods used. For example when  $200^{\circ}\text{F}$ . water was used with the expansion chamber closed and the liquid ammonia line open, the average temperature of four runs with respective volumes of rinse water of three, six and nine gallons was 53, 69 and  $80^{\circ}\text{F}$ ., respectively. Under the same conditions with the liquor line closed,

TABLE 8

*The effect of the amount of rinse water used to rinse an ice cream freezer on the temperature of the rinse water (expansion chamber and liquid ammonia line closed)*

Trial No.	Initial temp. of rinse water	Temperature of rinse water after running freezer for 2 minutes		
		3-gallon rinse	6-gallon rinse	9-gallon rinse
	°F.	°F.	°F.	°F.
1	60	34	34	36
	140	34	50	66
	180	44	67	80
	200	50	76	107
2	60	34	32	35
	140	35	50	65
	180	44	69	82
	200	51	80	110
3	60	34	34	36
	140	36	49	62
	180	47	69	82
	200	49	82	114
4	60	34	33	37
	140	35	50	68
	180	45	68	80
	200	50	78	112
Average of four runs	60	34	33	36
	140	35	50	65
	180	45	68	81
	200	50	79	111

the average temperature was 50, 79 and 111° F., respectively. These differences in temperatures are significant and show the value of plenty of rinse water and the proper handling of the freezers.

#### *Method of Calculating Bacterial Reductions.*

The method of calculating the percentage reduction in bacteria after each successive operation was as follows: The bacterial count of each rinse water was determined by plating in duplicate on Standard Milk agar. The average of the two counts per ml. for each rinse water was then totaled and the percentage bacterial reduction calculated by dividing the number per ml. found in each rinse water by this total. This gave the percentage of the total number removed by each operation in terms of bacteria per ml. The number per ml. found in the sanitized rinse water used to determine the number of bacteria left in the freezer after it had been sanitized was not used in the calculations.

#### GENERAL DISCUSSION

The data indicate that the preliminary treatment given to freezers is more important than the method used to sanitize them. When freezers are

properly and adequately rinsed, the bacterial load is reduced to a point where sanitization, by any of the three methods used in these experiments, is greatly facilitated. The advisability of using several rinse waters is also demonstrated since each successive rinse water greatly reduces the bacterial load. This is demonstrated in several instances in table 4 where the first rinse water removed as few as 73 per cent of the bacteria. Additional bacteria were removed by successive rinse waters until the number remaining was reduced in most cases to a satisfactory point by the sanitizing method used.

It should be noted in this connection that when the percentage reduction in the number of bacteria was small for the first rinse waters, the number remaining in the freezer after sanitizing was correspondingly large. This gives additional evidence for the necessity of adequate rinsing. Likewise, when the ice cream mix contained large numbers of bacteria, the number remaining after sanitizing was in many instances excessive. This was true despite the fact that the percentage reduction was large. The types of bacteria present would, of course, influence the percentage reduction which doubtless accounts for the exceptions found in some instances.

These data emphasize the necessity of an adequate and preferably an abundant supply of hot and cold water—a condition not possible in many retail ice cream manufacturing establishments such as soda bars, drug stores, restaurants and such places.

#### *Sanitizing Front and Rear Bearings.*

The results show that the rear bearing is harder to sanitize than the front bearing irrespective of the amount of water used or the method used to sanitize them. This does not bear out the assumption of Dalhberg and Marquardt (1) who state, "Although the rear bearings have not been considered in this study for obvious practical reasons, it is reasonable to assume that a procedure which sterilized the front bearings would also sterilize the rear bearings unless the front bearing is sterilized when the head of the freezer has been removed."

Steam was more effective than either hot water or chlorine in reducing the number of bacteria found in the bearings. This confirms the findings of the above authors (1) with respect to the front bearing. In the 8th edition (3) of Standard Methods for the Examination of Dairy Products, there is a new section entitled "Rinse Test as Applied to Ice Cream Freezers." The standard procedure recommended is 100-, 200- and 400-ml. portions of sterile rinse water for 10-, 20- and 40-quart freezers respectively. Freezers showing a total rinse count of 10,000, 20,000 and 40,000 for 10-, 20- and 40-quart freezers, respectively, are considered satisfactory. In these experiments, which were done before the standard procedure was formulated, one liter of sterile rinse water was used irrespective of the size of the freezer. However,



the bacterial content of the rinse water was determined on the ml. basis. The count per ml. was then multiplied by 1000 and this figure used as the basis of comparison with the bacterial counts considered satisfactory for ice cream freezers by the new standard methods.

On this basis, chlorine sanitization of freezers was best since 99.9 per cent of the rinse waters from chlorine sanitized freezers fell within the range considered satisfactory. Whereas, only 50 per cent of the rinse waters from steam sanitized freezers and 17 per cent of the rinse waters from hot water sanitized freezers fell within the satisfactory range.

However, when one considers the bacterial counts of the front and rear bearings, steam showed to better advantage than chlorine since the counts from the swabs were lower on the bearings of the freezers sanitized with steam than with chlorine. Hot-water sanitization was less effective than either steam or chlorine for both the freezer proper and for the front and rear bearings. The temperature data in table 8 show why hot water is the least effective since under ideal conditions the average temperature of 200° F. water as it entered the freezer was only 111° F. as it left it two minutes later.

It should be stated that the ice cream mixes used in these experiments had a bacterial count far in excess of what one would ordinarily find in commercial mixes. For this reason it is believed that the bacterial counts considered satisfactory in the 8th edition of Standard Methods for the Examination of Dairy Products (3) are reasonable and should be easily obtainable with any of the three methods used especially with chlorine and steam

#### *Significance of Escherichia coli Count.*

From the data presented in tables 4, 5 and 6, it is obvious that the *Escherichia coli* were killed in practically all instances as judged by plating of the sterile rinse water. They were found more frequently in both the front and rear bearings than in the freezer proper. This would indicate the difficulty of sanitizing the bearings. Likewise, they were more numerous in the rear than in the front bearing indicating that the rear bearing is more difficult to sanitize than the front bearing of an ice cream freezer.

#### SUMMARY

1. The first rinse water caused the greatest reduction in the number of bacteria in the freezer. Each subsequent washing reduced the number of bacteria left in the freezer but to a lesser degree.
2. The amount of water used to rinse the freezer is important since there is a direct relationship between the volume of water used and the number of bacteria remaining in the freezer.
3. The rear bearing is harder to sanitize than the front bearing of an ice cream freezer.

4. For sanitizing both the front and rear bearings, of a freezer, the order of effectiveness is steam, chlorine and hot water.

5. The valves to the liquid ammonia line and expansion chamber should be closed during the rinsing and sanitizing of a freezer to prevent dissipation of heat.

6. For most effective cleaning and sanitizing, freezers should be completely filled with water or at least to 90 per cent of their capacity.

7. As judged by the bacterial content of the sterile rinse water, chlorine was best, steam the next best, and hot water the least efficient method of sanitizing freezers.

8. There are marked temperature changes in water as it enters and leaves the freezer if the freezer is washed immediately after use. Under the most favorable conditions, hot water entering the freezer at 200° F. had a temperature of only 111° F. after it had been agitated two minutes. This explains why hot water sanitization is the least effective of the three methods.

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# AGE, LIVE WEIGHT AND MILK-ENERGY YIELD—A CORRECTION

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This correction relates to a previous article (1) in which initial live weight (live weight measured within 31 days after calving) was estimated by the New York chest-girth live-weight tape. Since that time a new girth-weight scale has been developed (2) for the specific purpose of estimating initial live weight, where age and breed are known. The New York tape is designed without regard to age, breed, or stage of lactation and as compared with the new scale it leads to gross systematic errors in the weight estimates (2).

The old weight estimates may be changed onto the new scale readily since both are based on chest girth. In the present paper this change has been made for those records with age reported and the age-weight-yield relations are re-examined. The yield data used are the eight-months partial lactation milk-energy yields in terms of pounds of four per cent milk per day (FCM).

## AGE, WEIGHT, YIELD

As before, the equation,  $FCM = a + bW - dA$ , is fitted to the observations at less than 7 years of age, with the results:

Holstein	Old scale, $FCM = 9.89 + .0128W + .96A$
	New scale, $FCM = 1.53 + .0217W + .54A$
Jersey	Old scale, $FCM = .48 + .0264W + .37A$
	New scale, $FCM = 3.80 + .0412W + .79A$

in which  $W$  = initial live weight in pounds and  $A$  = age in years.

The effect of removing the systematic errors in the New York tape has been to decrease the influence of age on FCM in the Holstein and increase it in the Jersey. In both breeds the effect of weight on FCM has been stepped up by about 60 per cent. The previous conclusion still holds that age independent of weight has only a negligible influence on yield. The influence of initial live weight, independent of age, is even more marked than before.

The mean weight on the new scale is practically the same as the mean weight on the old scale, in either breed but the range in weight is reduced on the new scale as compared with the old scale.

## AGE AND FCM/W

In figure 1 milk-energy yield per unit live weight ( $FCM/W$ ) is plotted against age. It is quite apparent that  $FCM/W$  is independent of age

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through the whole age range. A statistical verification of this is afforded by an analysis of variance with respect to 1000 FCM/W between and within age groups:

	<i>Holstein</i>	<i>Jersey</i>
Variance between age groups	48.4	31.5
Variance within age groups	38.0	38.4
F . . . . .	1.27	1.22
F, at 5% level	1.83	2.57

Clearly such differences as exist between age groups with respect to FCM/W are no more than might easily arise by reason of differences within age groups. The former conclusion remains: we may deal freely with FCM/W without regard to age of cow.

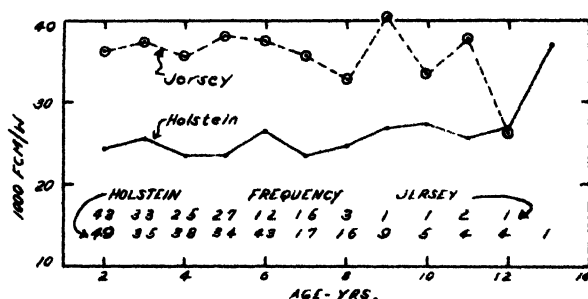


FIG. 1. Showing the change (or absence of change) in milk-energy yield per day per 1000 pounds live weight (1000 FCM/W) with age. There are no significant differences between the age groups with respect to FCM/W.

The independence of age (including all ages) and FCM/W is further tested by an analysis of covariance, taking account of a possible herd effect. This gives:

<i>Correlation</i>	<i>Holstein</i>	<i>Jersey</i>
Total . . . . .	+ .10	- .04
Between herds . . . . .	- .03	+ .10
Within herds . . . . .	+ .14	- .06

All of these correlations are below the five per cent level of significance, which together with their mixed nature in sign may be taken to mean that age and FCM/W are independent not only in the total population but also between and within herds. Age of cow may be ignored in FCM/W comparisons within a herd.

#### LIVE WEIGHT AND FCM/W

Analysis of covariance with reference to initial live weight (W) and milk-energy yield per unit initial live weight (FCM/W) gives the following:

<i>Correlation</i>	<i>Holstein</i>	<i>Jersey</i>
Total . . . . .	+ .06	+ .04
Between herds . . . . .	+ .02	+ .23
Within herds . . . . .	+ .08	- .01

All of these correlations are below the five per cent level of significance although in the Jersey breed the between-herds correlation is large enough to attract attention. It means, of course, that among the Jersey herds the herds with larger cows produce more milk-energy per unit live weight of cow than do the herds with smaller cows.

As compared to the old results a very important change is to be noted in the correlation between  $W$  and  $FCM/W$  within herds. *The present result shows that within herd and breed initial live weight and milk-energy yield per unit live weight are substantially independent.*

The correlation between  $W$  and  $FCM/W$  may be readily translated into the power equation,  $FCM = aW^b$ , if desired. In this power equation, as a close approximation,

$$b = 1 + r_{W(FCM/W)} V_{FCM/W} / V_W$$

where  $V$  indicates standard deviation divided by mean. Applying this formula it comes out that in the Holstein breed, and within herd,  $FCM$  is proportional to  $W^{1.02}$ ; while in the Jersey breed, and within herd,  $FCM$  is proportional to  $W^{.95}$ . If it is accepted that the within herd correlations represent some degree of uniformity of environment, the present results give important support to the theory that milk-energy yield tends to be proportional to initial live weight. However, the disparity between the two breeds with respect to  $FCM/W$  remains as before (2).

The correlation between  $W$  and  $FCM$  may be readily derived from the correlation between  $W$  and  $FCM/W$  by the formula:

$$r_{WFCM} = \frac{V_W + r_{W(FCM/W)} V_{FCM/W}}{[V_W^2 + V_{FCM/W}^2 + 2r_{W(FCM/W)} V_W V_{FCM/W}]^{.5}}$$

Applying this formula the within herd correlation between  $W$  and  $FCM$  is +.44 in the Holstein records and +.55 in the Jersey records.

If the correlation between  $W$  and  $FCM/W$  is zero, the above formula reduces to terms of variability ( $V$ ) in  $W$  and  $FCM/W$ , and if  $V_W = V_{FCM/W}$  the expected correlation between  $W$  and  $FCM$  is .71. Within a herd and breed variability in live weight ( $V_W$ ) is not apt to exceed variability in energy yield per unit live weight ( $V_{FCM/W}$ ) and hence the correlation between live weight and energy yield is not likely to exceed +.7.

#### DISCUSSION

The importance of having an estimate of live weight of the cow in connection with the estimate of her milk-energy yield is very evident. The estimate of yield under present systems may be regarded as satisfactory (although hardly precise). The estimate of live weight under present systems is not at all satisfactory. In the first place the live weight estimate is usually entirely absent—which may be better than badly erroneous estimates, or estimates made at indiscriminate stages of lactation.

An instance of the effect of systematic errors in the live weight estimates

has been noted above. Thus the estimate of live weight during the first month of lactation by the New York tape leads to the result that FCM is proportional to the  $\frac{2}{3}$  power of live weight (1, footnote 3) while the estimate of live weight by the Nebraska-Illinois tape (2) for the same cows and FCM records leads to the result (above) that FCM is proportional to the first power of live weight. One result supports the  $\frac{2}{3}$  power theory, the other supports the direct proportionality theory.

The basis of the difference in the two scales of estimating live weight from chest girth has been shown previously (2, figure 3). That it should lead to such a difference in the philosophical interpretation of the relation of live weight to yield emphasizes the necessity of *accuracy* in the estimate of live weight and the avoidance of systematic errors. Of no less importance is the *stage of lactation* at which live weight is measured (3). The confusion introduced by measuring live weight at indiscriminate stages of lactation may completely obscure the essential relation between size of cow and milk-energy yield.

#### SUMMARY

The correction of now known systematic errors in the estimate of live weight as previously used (1) shows the influence of weight on yield, independent of age, to be greater than previously found. The influence of age on yield, independent of weight, is negligible, as before.

Milk-energy yield per unit live weight is independent of age of cow, whether within herd, between herds or in total.

Within herd and breed (Holstein, Jersey) energy yield per unit live weight is independent of live weight. Or, if energy yield is expressed as a power function of live weight the within herd relation shows milk energy proportional to the 1.02 power of live weight in the Holstein records and proportional to the .98 power of live weight in the Jersey records.

Two essential points in the live weight measurements are stage of lactation and accuracy (avoidance of systematic errors). The present results are based on live weight measured within 31 days after calving.

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# A TEST FOR THE PROTEIN STABILITY OF MILK

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The stability of the protein of milk is a matter of great importance in many phases of the dairy industry. Frequently an excessive loss of protein stability is the chief factor limiting the extent to which some treatments may be applied to milk or milk products. The problem is often of concern in the processing of such products as evaporated milk, ice cream, cream and some types of modified fluid milk.

It is not the purpose of this paper to enter into a lengthy review of protein stability since more adequate treatment of the subject may be found elsewhere (1, 2). The physical and chemical aspects of the problem will be mentioned only briefly. Fundamentally, the degree of stability of the milk proteins is dependent upon the hydration and the charge of the particles. These factors are influenced in turn by temperature, reaction, salts, previous heat treatment, or the action of other dehydrating or denaturing substances. The controlled application of one or more of these factors has been the basis for most methods of measuring protein stability.

One of the most widely used methods of studying protein stability has been that of subjecting the milk to high temperature under pressure in a manner simulating the sterilization of evaporated milk. The use of pilot sterilizers by condenseries is a routine operation for determining the degree of stability and the corrective measures needed. The technique of such tests is described by Hunziker (2). The same general methods have been applied by many investigators in studies of heat stability (3, 4, 5, 6, 7, 8). The use of a pilot sterilizer or similar equipment makes it possible to duplicate on a small scale many of the processing operations which affect stability, particularly with respect to the ability of the product in question to withstand sterilization. While the method is largely of value for the control and study of evaporated milk and related products, it is not well suited for use with many of the other dairy products.

Another method of measuring the coagulability of milk is the alcohol test (9). The test was originally devised as a measure of acidity but has been shown by Sommer and Binney (10) to be of little practical value due to the influence of the salts and other milk constituents. The test has been used for the detection of milk lacking stability during sterilization but there is some disagreement as to its accuracy for this purpose (2).

In 1935 Keith and Freeman (11), to determine the amount of HCl required to produce flocculation, employed an acid coagulation test which consisted of adding varying concentrations of N/40 HCl in distilled water to 5 ml. of ice cream mix.

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Dahle and Rivers (12), in a study of ice cream, used a modified alcohol test essentially the same as the acid coagulation test of Keith and Freeman. The samples were observed for flocculation after the addition to the ice cream mix of varying concentrations of alcohol in distilled water instead of HCl.

Another method of estimating protein stability is a determination of the coagulating time with rennin. Mattick and Hallett (13) and others (6) have employed this technique in studies of heat stability.

In 1931 Ramsdell, Johnson and Evans (14) proposed a phosphate test for the detection of milk unstable to heat. After the addition of 0.5 M mono-basic potassium phosphate to 2 ml. of milk and mixing, the tubes were immersed in boiling water for five minutes, then cooled and examined for coagulation. A low "phosphate number" indicated low heat stability.

Various other methods of studying protein stability have been tried including such general tests as titratable acidity, pH measurements both with and without the use of coagulating agents, and simple boiling tests. These methods have been of little value except for some limited and specific applications, probably because results were too often influenced by factors having little or no relationship to protein stability.

The writer has been interested in the development of a process for modifying milk by the addition of a proteolytic pancreatic enzyme. One of the problems was a control system which would insure proper treatment of the milk and at the same time would be a simple and inexpensive routine for milk plants. Preliminary investigation indicated that the method best suited for the purpose was one involving a determination of protein stability and particularly with respect to the ability of the milk to withstand boiling without coagulation. A number of procedures were attempted before a test was developed which was both simple and accurate. A description of the test follows.

#### METHOD

This test for protein stability includes mixing increasing amounts of N/10 HCl with portions of the milk, boiling the mixtures for a specified length of time and then examining the samples for coagulation. While originally devised as a control test for the enzymatic treatment of milk, it need not be limited solely to that application.

#### *Equipment and reagents for the test:*

- 10-ml. volumetric pipette
- 1-ml. pipette, graduated in 0.1 or 0.05 ml.
- Supply of test tubes, 16 × 150 mm. (Pyrex)
- Test-tube rack
- Water bath
- N/10 hydrochloric acid

**Procedure:**

Arrange and number a series of test tubes as follows, adding N/10 HCl to each by means of the 1-ml. pipette in the amounts shown:

<i>Tube no.</i>	N/10 HCl (ml.)
0	0.00
5	0.05
10	0.10
15	0.15
20	0.20

etc., as needed

(Note: HCl in the amounts 0.05, 0.15, 0.25 ml., etc., can be estimated satisfactorily with a pipette graduated in 0.1 ml.)

The tube numbers correspond to  $100 \times$  the ml. of N/10 HCl added to each tube. This eliminates the decimal point as well as any need for further interpolation of results.

Add to each tube by means of a volumetric pipette 10 ml. of the milk to be tested. All tubes are then placed in a water bath maintained at the boiling temperature. After 10 minutes the tubes are removed from the boiling water and examined for coagulation by tipping. The number of the first tube in the series which shows coagulation represents the end-point and is recorded as the stability number of the milk.

**RESULTS**

In applying this test to a variety of untreated fresh milks a range in stability of from 40 to 100 has been observed. A frequency table of these results is shown in table 1. Samples with extremely high or low stability

TABLE 1  
*Distribution of stability numbers of untreated fresh milk*

Stability number	No. of times observed
40-49	2
50-59	15
60-69	32
70-79	41
80-89	11
90-99	3
100-109	1
Total observations	105
Ave. stability number	66.6

numbers have not been found to be common. The average stability number has been found to be about 60 to 70. Results have been consistent and

properly stored milk has maintained the same stability number for several days.

It was observed in some of the early work with this test that pasteurization seemed to increase the stability number of milk. Consequently, an experiment was run to determine more exactly what effect could be expected. The samples for this investigation were pasteurized in glass bottles in the laboratory. Preheating periods of 10, 20 and 30 minutes were used in conjunction with the usual holding process at 143–145° F. for 30 minutes. The data from this study are shown in table 2. Pasteurization increased the

TABLE 2

*The effect of the preheating time upon the stability number of pasteurized milk*

Raw milk	After pasteurization		
	Time of preheating		
	10 min.	20 min.	30 min.
50	60	55	60
70	80	80	85
65	75	75	75
70	85	85	90
60	70	70	70
60	75	80	80
50	60	60	55
70	80	80	80
65	75	75	75
65	75	75	75
Ave. 62.5	73.5	73.5	74.5

stability number of the milk slightly more than 10. In some cases the stability number increased as the length of the preheating period was increased. However, in the samples tested the average increase in the stability number as a result of longer preheating periods was negligible.

There was also an opportunity for a limited study of the effect of copper contamination upon protein stability. Known amounts of copper, in the form of copper sulfate, were added to the milk and the effect upon the stability number observed. As shown in table 3 copper contamination de-

TABLE 3

*The effect of copper upon the stability number of milk*

Copper added, in p.p.m.			
0.5	1.0	2.0	3.0
Decrease in stability number			
.....	10	15	20
.....	10	15	.....
5	10	.....	.....
Ave. 5	10	15	20

creased the stability of the milk, the loss of stability becoming greater as the amount of copper was increased.

In the application of this test to the enzymatic treatment of milk it is the general practice to control the amount of enzyme activity so that the stability of the milk is reduced to within the limits of from 20 to 40. This insures adequate treatment and at the same time provides a satisfactory margin against coagulation upon subsequent boiling.

With respect to simplicity the method is largely satisfactory. The apparatus and reagents are standard items which can be procured easily and inexpensively if not already on hand. A minimum of technical knowledge is necessary for proper performance of the test.

#### SUMMARY

A simple test for protein stability is described which includes mixing increasing amounts of N/10 HCl with portions of the milk, boiling the mixtures for a specified length of time and examining the samples for coagulation.

The stability number of untreated fresh milk has been found to average about 60 to 70 as indicated by the test.

Pasteurization tends to increase the stability of milk.

Copper contamination tends to lower the protein stability of milk.

#### ACKNOWLEDGMENT

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# THE EFFECT OF NATURAL MILK ENZYMES, ACID, AND SALT UPON THE KEEPING QUALITY OF BUTTER STORED FOR SIX YEARS

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In a previous publication, Guthrie, Scheib, and Stark (5) reported the results of their studies on the effect of certain factors upon the keeping quality of butter held for 36 days at 50° F. (10° C.). Other temperatures which would also permit the growth of microorganisms were tested. Quality was measured by scoring the butter on the basis of taste and odor. This measure of quality was used because it is recognized that the consumers' response to the flavor of butter largely determines its commercial value.

*Pasteurization temperatures.* It was found that pasteurization temperatures equivalent to 165° F. (73.9° C.) for 30 minutes were required to destroy the harmful natural enzymes of milk. It was suggested that a flash pasteurization of 200° F. (92.5° C.) or higher would probably be the equivalent of 165° F. for 30 minutes. Neither heating the cream to 145° F. (62.8° C.) nor 155° F. (68.3° C.) for 30 minutes was found to be sufficient to inactivate completely the harmful natural enzymes of milk. In our early preliminary studies on pasteurization temperatures, 180° F. (82° C.) for 30 minutes was tested. This temperature of pasteurization resulted in butter having noticeably cooked flavors with no improvement in keeping quality over butter made from cream pasteurized at 165° F. for 30 minutes. The reports by Kende (7) and Guthrie and Brueckner (4) of their studies on oxidized flavors in pasteurized milk, indicated the importance of these higher temperatures of pasteurization. In a personal communication, dated March 16, 1937, Mr. C. W. Fryhofer, Supervising Federal-State Grader of Minneapolis, Minnesota, mentions the results obtained by the Land O'Lakes Creameries as follows: "We found a decided improvement in the keeping quality of the butter when the pasteurization temperature was raised to 165 degrees and the cream held for thirty minutes at that temperature before cooling. When the cream was held for only fifteen minutes at a temperature of 165 the results were not satisfactory." Jensen (6), Wiley (11), Wilster (12), and Fabricius and Bird (2) have since reported that higher pasteurization temperatures than previously used definitely improved the keeping quality of butter.

*Bacteria.* It is not possible to evaluate correctly the part played by bacteria in the spoilage of butter made from raw cream since the action of

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the harmful natural enzymes of milk partially or completely overshadow the effect of the bacteria present. It is possible to determine unmistakably the effects of certain harmful bacteria capable of growing in butter made from cream pasteurized at 165° F. for 30 minutes, since the natural enzymes of milk have been destroyed by this pasteurization process. Our preliminary studies, the object of which was to determine the part played by bacteria in the spoilage of butter, convinced us that a total count of the number of bacteria in butter meant little or nothing with regard to its keeping quality or the part played by bacteria in its deterioration. Stark and Scheib (10), in a study of bacteria growing in butter to numbers sufficiently large to be of significance in its keeping quality, if other important spoilage factors were absent, found that fat-splitting and casein-digesting, gram negative, non-spore-producing rods were the types usually predominant in butter of poor quality. Flake and Parfitt (3) have also reported that large numbers of rod-shaped organisms were generally associated with butter of poor keeping quality. Our tests showed that pasteurization of cream at 165° F. for 30 minutes destroyed these types of bacteria. However, in making experimental butter, difficulty was encountered in avoiding recontamination with these types of bacteria in cream pasteurized at such temperatures. It was suggested that the possible importance of the recontamination of commercial butter by these harmful types of bacteria should be recognized. Data presented on lots of sweet cream-unsalted butter made from cream pasteurized at 165° F. for 30 minutes and held for 36 days at 50° F. showed the uncontaminated butter to have a score of 92 at the end of this storage period, while lots of butter contaminated with these harmful types of bacteria had a score of 83.

*Acid and salt.* In a previous publication (Guthrie, Scheib and Stark, 5), it was stated: "In the presence of certain microorganisms and at a temperature at which they can grow, the *preserving* action of acid and salt is well known." . . . "The presence of either acid or salt in butter, not containing other spoilage factors considered in this study, resulted, after storage, in a *poorer quality* butter." "The combined *deteriorating* effect of both acid and salt was shown." Wiley (11) and Bendixen (1) have since confirmed the importance of salt and high acidity as deteriorating factors on the keeping quality of butter.

Rogers, *et al.* (9) made the following statement: "The most serious difficulty in experimental work on butter is in controlling the conditions under which butter is made. *So many apparently unimportant factors have an influence on the flavor that it is nearly impossible to make butter with a normal flavor and have only one varying factor.* The work is further complicated by the sequence of flavors that frequently occurs in butter held in storage. It is evident that the usual off flavors are in many cases a combination of flavors and that the flavors themselves are caused by a combina-

tion of circumstances and not by a single cause. It is probable also that identical flavors may be caused by different factors."

In all of our studies the aim has been to conduct experiments in such a manner that it would be possible to observe the quality of butter: first, in the absence of any of the deteriorating factors considered by us; second, in the presence of but one of each of these deteriorating factors; and third, in the presence of more than one of these deteriorating factors. Although commercial butter is not and cannot be made under such carefully controlled conditions, it is of extreme importance to know the combined significance of these factors and their relative importance as they affect butter quality.

All butters in each series of churnings were made from the same fresh cream of high quality. The extremely low bacterial content of the raw cream and the freshly made sweet cream-unsalted butter indicated the high quality of the cream used. A number of duplicate sets from five different series of butters were placed at different holding temperatures. This paper is a report on the influence of: pasteurization temperatures, milk enzymes, acid, and salt, in the absence of bacterial action, on the keeping quality of the duplicate sets of butter stored for 6 years at 0° F. (-17.8° C.) to -10° F. (-23.3° C.).

After 6 years of storage at 0° F. to -10° F., the duplicate samples of butter of each type for each series were scored for taste and odor, and total and differential bacterial counts were made. Other duplicate sets were held for one week at 50° F. for a further check on the action of enzymes, bacteria, salt, and acid, if these agents were present, on the keeping quality of such butter, when held at a temperature similar to that at which butter would normally be held under commercial conditions when removed from storage. The bacterial content of all the duplicate lots of butter, whether or not they were held for one week at 50° F., were all too low, from less than 1 to a few hundred per gram, to have been of any significance in the quality of the butter. These results are in accordance with what one would have anticipated, since it is well known that bacteria cannot grow in a frozen medium, and that the bacteria present in such an environment gradually die.

The results obtained from the storage of these lots of butter for six years at 0° F. to -10° F. are summarized in table 1. The judges in reporting their scores on the sweet cream-unsalted butters made from cream pasteurized at 165° F. for 30 minutes indicated these butters to have what is known as a slight storage flavor, indicating that these butters had lost only their original ideal freshness. This is the explanation offered for the decrease in score from 95 to 92.3 during this storage period. Using 92.3 as the score for butter which had present none of the deteriorating factors considered in this study, it is readily observed that the presence of salt in butter is harmful to its keeping quality but less harmful than is acid, or acid and



TABLE 1

*The influence of pasteurization temperatures, milk enzymes, acid, and salt, in the absence of bacterial action, on the keeping quality of butter stored for six years at 0° F. to -10° F. (-17.8 to -23.3° C.)*

No. of series of churnings	No. of samples	Description of butter	Average score of fresh butter	Average of score after storage for 6 years at 0 to -10° F. (-17.8 to -23.3° C.)	Average score on 6-year-storage butter after holding 1 week at 50° F. (10° C.)	Decrease in average score of 6-year-storage butter after holding 1 week at 50° F. (10° C.)
Butter made from cream pasteurized at 165° F. (73.9° C.) for 30 minutes						
5	10	Sweet—unsalted	95	92.3	92.3	0.0
5	10	Sweet—salted	95	90.8	90.8	0.0
5	10	Sour—unsalted	95	87.5	87.0	0.5
5	10	Sour—salted	95	85.4	84.6	0.8
Butter made from cream pasteurized at 145° F. (62.8° C.) for 30 minutes						
5	10	Sweet—unsalted	95	90.4	88.6	1.8
5	10	Sweet—salted	95	88.4	87.2	1.2
5	10	Sour—unsalted	95	86.4	85.8	0.6
5	10	Sour—salted	95	84.8	84.2	0.6
Butter made from raw cream						
2	4	Sweet—unsalted	93.5	83.0	83.0	0.0
2	4	Sweet—salted	93.5	83.0	83.0	0.0
2	4	Sour—unsalted	93.5	85.0	84.0	1.0
2	4	Sour—salted	93.5	83.0	83.0	0.0

The flavor of the pasteurized cream butters, with the exception of the 165° F. sweet cream-unsalted butters were all oily or tallowy. The raw sweet cream butters were all rancid-bitter; the raw sour cream butters were all oily or tallowy. The butters were scored by E. S. Guthrie, W. E. Ayres, and B. J. Scheib. Many samples, probably, should have been scored below 83. This figure, however, was as far from the thresholds of smell and taste as the judges dared to venture.

salt combined. The scores 90.8, 87.5, and 85.4 indicate the proportional deteriorating effect of salt, acid, and salt and acid on the keeping quality of butter stored at 0° F. to -10° F. for 6 years when examined immediately after removal from storage. The scores obtained on duplicate samples of these butters after an additional one week of holding at 50° F. further indicate hastened deterioration of these butters by acid. In the absence of harmful natural milk enzymes or spoilage bacteria, the lowered score of the sweet cream-salted butter, which is 1.5 points less than the sweet cream-unsalted butter, and the lowered score of the sour cream-unsalted butter which is 4.8 points less than the sweet cream-unsalted butter, and the decrease in score of the sour cream-salted butter which is 6.9 points less than the sweet cream-unsalted butter, indicate roughly the relative value of salt and acid as spoilage factors in storage butter. The spoilage relationships, according to these results, appear to be approximately—salt is to acid as one is to three (1:3), or that acid causes about three times as much decrease in the butter score as is caused by salt. As a possible indication that the relationships hold and are also cumulative, it may be observed that acid and

salt together showed approximately four times as much decrease in score as did salt alone. These findings seem to be significant and tend to confirm the statement made by Rogers, *et al.* (8): “. . . the change in the pasteurized ripened-cream butter stored at 0° F. was four times as great as that in the pasteurized sweet-cream butter at the same temperature, . . .”

The difference between the scores of sweet cream-unsalted butter, 92.3, for butter made from cream pasteurized at 165° F. for 30 minutes, and 83 for the sweet cream-unsalted butters made from raw cream shows a difference in score of 9.3 points which may be attributed to the action of harmful natural milk enzymes. To continue further the analogy previously suggested, this indicates that the ratio of salt is to acid, is to natural milk spoilage enzymes, as one is to three, is to six (1:3:6). The scores obtained on sweet cream-unsalted butters made from cream pasteurized at 145° F. for 30 minutes indicate that this degree of pasteurization is roughly only 80 per cent effective. The continued and more rapid deterioration of the sweet cream butters made from cream pasteurized at 145° F. for 30 minutes when the butters were held an additional week at 50° F. also indicates the failure of this degree of pasteurization to inactivate effectively the natural spoilage enzymes of milk.

#### SUMMARY

The effect of harmful natural milk enzymes, acid, salt, and acid and salt upon the keeping quality of butter held at 0° F. (-17.8° C.) to -10° F. (-23.3° C.) for 6 years, has been studied. Five series of butters consisting of 192 samples made and held under known and carefully controlled conditions have been examined.

Pasteurization of cream at 165° F. (73.9° C.) for 30 minutes inactivates the harmful natural milk enzymes; whereas pasteurization of cream at 145° F. (62.8° C.) for 30 minutes is apparently only about 80 per cent effective.

The presence of either acid or salt in butter, not containing other spoilage factors considered in this study, resulted, after storage, in a poorer quality butter. The relationship appears to be salt is to acid as one is to three (1:3).

The combined deteriorating effect of acid and salt was shown to be approximately four times as great as the harmful effect of salt alone.

It appears that the relationship between the spoilage factors of salt, acid, both acid and salt, and the natural milk-spoilage enzymes, is approximately one is to three, is to four, is to six (1:3:4:6).

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# IDENTIFICATION OF THE WHITE PARTICLES FOUND ON RIPENED CHEDDAR CHEESE\*

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When canned natural cheddar cheese had ripened for 5 to 7 months, white particles about the size of a small pin head began to appear on the surface and throughout the body of the cheese, especially along the cracks between the curd particles. They also appeared quite heavily on both sides of the parchment paper in which the cheese was wrapped. Similar granules have been observed by other authors from time to time on various types of natural cheese but reports as to their identity have been quite varied and conflicting. These particles are very common in well ripened cheddar cheese and have undoubtedly been submitted to many examinations not reported in the literature.

In 1909, Van Slyke and Publow (11) mentioned the formation of white particles in and on cheddar cheese ripened at low temperatures, and, after partial analysis, concluded that they were probably calcium soaps; calcium combined with some of the higher fatty acids.

Dox (2) described the occurrence of these white particles on the surface and especially in the crevices between the curd particles of Roquefort cheese. Chemical tests and microscopical examination of the recrystallized material indicated that it was composed largely of tyrosine.

More recent investigations have shown the particles to be calcium lactate. X-ray analysis of the white specks isolated from well ripened cheddar cheese, according to Tuckey, Ruehe, and Clark (10), showed them to be calcium lactate. Then McDowall and McDowell (7) isolated 0.9 gram of the particles and subjected it to chemical analysis. They found it to be calcium lactate with 11.3 per cent of protein and 17.2 per cent of fat present as cheese adhering to the specks.

## EXPERIMENTAL RESULTS

The description of the physical appearance of the material found by different authors seemed the same, but three different chemical entities are reported. Of course it is conceivable that the chemical composition may vary somewhat under different conditions. It would be rather surprising.

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however, if a compound as soluble as calcium lactate should accumulate in sufficient quantities to be precipitated in a normal cheese.

Ordinarily the material is difficult to collect in any quantity without contaminating it with particles of cheese. However, in canned cheese collection of the material was fairly easy for much of it would collect on the surface of the parchment paper. The particles could be obtained from the paper free from any insoluble cheese particles by allowing the wrapper to dry and scraping the material off with a spatula, or by washing off with cold water any cheese particles adhering to the surface of the wrapper and extracting the material with boiling water for a few minutes. The material was recovered by evaporating until the solution was highly concentrated and finally dried over concentrated sulphuric acid. In the course of several months a composite sample from several batches of canned cheddar cheese was collected on which chemical analyses were made in an attempt to identify the material.

The material was quite insoluble in cold water but was slightly more soluble in boiling water, a fact which indicated that it contained little or no calcium lactate. A qualitative test for lactic acid, as described by Troy and Sharp (9), was made using a 0.1 gm. sample. Another sample of the material to which some calcium lactate had been added served as the control and was treated in the same way. The test for lactic acid on the control was strongly positive while the test on the white material alone was almost completely negative. Against a white background there was a slight indication of a pink color which developed after 15 to 20 minutes, standing. According to Troy and Sharp, this test will detect 0.002 per cent lactic acid, beginning with 125 cc. of milk of which about 65 gm. were finally used as filtrate, or about 1.3 mg. of lactic acid. Assuming the same sensitivity, 1.3 mg. would be equal to 1.3 per cent lactic acid when 0.1 gm. sample was used. The weakness of the test, however, showed that if any lactic acid were present it was present only in minute traces as an incidental contaminant. The test for calcium with ammonium oxalate was positive though not very strong. These tests eliminated the possibility of calcium lactate as an important constituent.

Certain other qualitative tests were also made on the solution of the material, among them the Biuret test for proteins which was negative. The Xanthoproteic test was strongly positive as well as the reaction with Millon's reagent. These results pointed strongly in favor of tyrosine.

A sample of the material was found to contain 1.57 per cent ash which was far too low for calcium lactate. The ash was further analyzed for calcium and phosphorus. The calcium content amounted to 40.19 per cent, expressed as CaO while the phosphorus made up 38.94 per cent, expressed as  $P_2O_5$ . These results would indicate that calcium phosphate salts were present as an impurity or as a minor constituent of the compound.

A portion of the material was also analyzed for nitrogen by the Kjeldahl method and duplicate determinations showed that 7.63 and 7.57 per cent nitrogen was present. Correcting this value to allow for the ash the nitrogen content of the combustible material was 7.72 per cent. The theoretical value for tyrosine is 7.73 per cent nitrogen.

The next step was to purify some of the material and identify it in pure form. This was accomplished by dissolving the material in hot water and recrystallizing three times. Microscopic examination of the crystals showed them to be long white needle-like crystals in the characteristic sheave-like arrangement of tyrosine. The melting point of these crystals was 293° C. but after two more recrystallizations from water it was 310° C. No further purification seemed necessary although the melting point of tyrosine is around 315° C. as reported by Cole (3). Cole states that tyrosine is laevo-rotatory in aqueous solutions.

As a final identification of the material the dibenzoyl derivative was made by adding benzoyl chloride to an aqueous solution containing sodium bicarbonate and some of the purified material from the cheese wrapper. The purpose of the sodium bicarbonate was to keep the reaction mixture weakly alkaline during benzoylation.

The procedure was as follows: 0.1 gm. of l-tyrosine or purified cheese material was placed in 75 ml. of distilled water with 5 gm. of sodium bicarbonate and the mixture warmed until complete solution was obtained. It was then cooled to 20° C. or below and 2.5 ml. of benzoyl chloride was added slowly with shaking. The mixture was placed in the cold room (4-7° C.) for at least an hour after which it was acidified with hydrochloric acid, allowed to stand for half an hour and then filtered. The precipitate was washed with water, air dried, and the benzoic acid removed by extraction with warm petroleum ether.

Several solvents were tried on the precipitate remaining after the petroleum ether extraction. Dibenzoyl tyrosine did not crystallize well from glacial acetic acid. Chloroform dissolved the dibenzoyl tyrosine readily and it could be completely precipitated by adding about an equal volume of petroleum ether, but the material seemed to precipitate in an amorphous form rather than yielding good crystals. Absolute ethyl alcohol gave the best results. The dibenzoyl tyrosine was dissolved in hot absolute alcohol and when completely in solution, water was added dropwise until a slight opalescence developed. Needle-like crystals formed on cooling. After several recrystallizations from this solvent the dibenzoyl tyrosine was obtained pure. The melting point of dibenzoyl tyrosine according to Abderhalden and Brockman is 216-217° C. (1). Dibenzoyl tyrosine prepared from chemically pure tyrosine and benzoyl chloride by the above method had a melting point of 216.8° C. (corrected). The melting point of the product made with the white material from cheese and benzoyl chloride was

also  $216.8^{\circ}\text{C}$ . (corrected). The melting point of a mixture (50-50) of pure dibenzoyl tyrosine and the unknown was  $217.0^{\circ}\text{C}$ . (corrected). Since the mixed melting point remained unchanged, the unknown compound prepared from the purified white cheese particles must have been dibenzoyl tyrosine and the white particles obtained from the cheese wrapper must have been largely tyrosine.

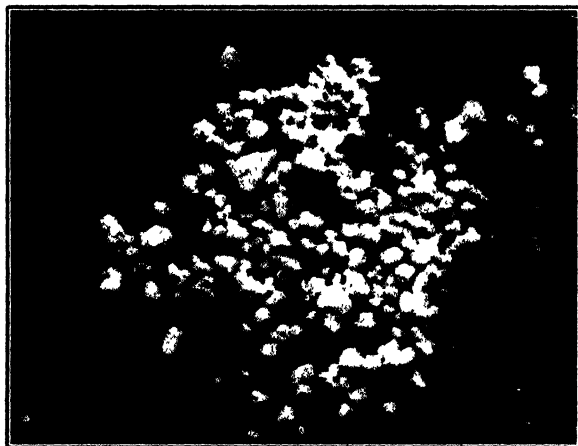


FIG. 1. The white particles as removed from the parchment wrapper on the canned cheddar cheese.  $\times 30$ .



FIG. 2. The sheath-like crystals typical of tyrosine prepared by recrystallization of the white particles from aqueous solution.  $\times 45$ .

Photomicrographs (figs. 1 and 2) were taken of some of the granules showing them as they appeared respectively after they were scraped from

the parchment wrapper with a spatula and after they had been purified by recrystallization from water.

#### DISCUSSION

The white particles so often observed in well ripened cheese have been reported as calcium soap, tyrosine, and calcium lactate. It seems doubtful that such different results could be obtained on the same material so there is the possibility of several types of white particles. However, a discussion of the various results may aid in interpreting previous findings.

Van Slyke and Publow (11) found calcium and no phosphorus. The particles were not especially gritty and smeared readily between the fingers in a greasy manner. They then concluded the particles were calcium soap. Dox (2) found the particles burned on platinum wire without melting and with no ash. He secured a strong Millon reaction and Piria's test. Recrystallization gave typical tyrosine crystals so he concluded the particles were tyrosine. The observed facts in these two investigations are not contradictory so far as they duplicate each other. The observations in the present study agree with them except that small amounts of phosphorus were found and Piria's test was not made.

It is difficult, however, to reconcile these findings with those showing the particles to be calcium lactate. Since the particles are crystallized from a solution containing calcium lactate there must be traces of calcium lactate as impurities on the particles. This may explain the X-ray pattern of calcium lactate secured by Tuckey, Ruehe, and Clark (10). Such impurities cannot explain the data of McDowall and McDowell (7) who found 35.1 per cent lactic acid in the particles. Either two different types of particles are involved or gross contamination or errors occurred.

Certain facts tend to indicate against the particles being calcium lactate. According to Suzuki, Hastings, and Hart (8) the lactic acid content of cheese was high within 3 days and increased for 3 to 5 months. It was lowest in 10-month-old cheese. This means that calcium lactate particles should appear promptly and gradually disappear after 5 months. Actually they begin to appear in 5 to 7 months and increase with increased age of cheese.

Then there is the question of solubility of calcium lactate. The highest concentration of lactic acid reported by Suzuki, Hastings, and Hart (8) amounted to 3.1 per cent calcium lactate in the water present in cheese. The solubility of calcium lactate with 5 molecules of water of crystallization varies from 3.0 per cent at 0° C. to 7.3 per cent at 30° C., Hodgman (4). These solubilities must mean that particles picked from cheese at room temperature should be dissolving into the cheese as the cheese is not saturated with calcium lactate at room temperature. Actually this solution does not occur for the particles are insoluble even when rinsed with water.

Tyrosine is a normal constituent of well ripened cheese, and was first isolated and recognized by Liebig in 1846-47 (5, 6). Since it is practically



insoluble in cold water (1 part in 2500 of water at room temperature) its precipitation from cheese ought to be expected. In the present investigation all results indicated the particles were tyrosine.

#### CONCLUSIONS

Chemical analyses were made to determine the identity of the white particles appearing in ripened cheddar cheese. The insolubility of the material in water, its low ash content, and a negative test for the lactate radical eliminated the possibility that the material was calcium lactate. The large amount of calcium and phosphorus present in the ash suggested a calcium phosphate salt as an impurity or a minor constituent of the white material.

The chemical reactions of the material were all characteristic for tyrosine. It was very insoluble in cold water but fairly soluble in boiling water. Tests for the hydroxyphenyl group were positive and the nitrogen content corresponded with the calculated value for tyrosine. The crystal formation was characteristic for tyrosine and the melting point was only slightly below the value reported for this amino acid. Confirmatory proof was obtained by the melting point of the dibenzoyl derivative.

The results of these tests seemed sufficient proof that the white material was principally tyrosine.

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# SUPERHEATED SOFT CURD MILK\*

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A careful review of the literature dealing with the use of modified cow's milk as a substitute for breast milk in the feeding of infants (particularly the new born) leads inescapably to the conclusion that the most satisfactory types of milk for the purpose are those which have undergone an evident degree of precoagulation in the modifying process. Examples of such milks are: acidified milk, evaporated milk, boiled milk, dried milk and some of the proprietary powdered preparations. The first two types mentioned have undergone perceptible coagulation and almost invariably exhibit curd tension values of zero. Boiled milk and dried milk while not perceptibly coagulated in most cases, have suffered some degree of protein denaturation and loss of "soluble nitrogen" which may be looked upon as the first stages of such a coagulation. Such milks are not always reduced to zero curd tension values but rarely give readings in excess of five or six grams.

The dairy industry has produced for years a product which undergoes a very evident protein coagulation, induced by heat, in the process of manufacture. This product is known in dairy circles as superheated plain condensed milk. On a reconstituted fluid basis, it should have a zero curd tension value; it should produce a very fine curded structure under peptic digestion conditions; and, judging from results secured with acidified milk and evaporated milk, it should prove equally as suitable for the feeding of infants.

In view of the fact that so many radically treated forms of milk have been advanced as more digestible soft curd types, presumably useful in infant feeding, it was felt that reconstituted superheated milk, a form which results from a process long established in the industry should be studied and described. The investigation presented here was accordingly undertaken.

## METHODS

Fresh fluid whole milk was analyzed for solids, preheated at 145° F. for 30 minutes, drawn into a small vacuum pan and concentrated by removing water until a condensation ratio of approximately three to one was obtained as indicated by a Baume hydrometer. The product was drawn from the pan and analyzed for solids. Then, together with a portion of the original fluid milk, the concentrated milk was superheated in a hot water bath or by

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means of a heating coil at temperatures varying from 194° F. to 205° F. (in different trials) for periods of time necessary to obtain the desired thickening or the desired degree of protein coagulation. The time required varied from about 10 minutes to about 30 minutes. The fluid and concentrated portions of the milk were then set in cold water and cooled to avoid further thickening or coagulation. Portions of the fluid preheated milk, the fluid milk heated to the superheating temperature, the unsuperheated concentrated milk, the concentrated superheated milk, the unsuperheated concentrated reconstituted milk and the superheated concentrated reconstituted milk were homogenized at a pressure of 3000 lbs. and cooled to 40° F. Samples of the same lots of milk were also taken and held for examination in the unhomogenized state. Reconstitution was accomplished by diluting the concentrated product with water to the original solids content.

Curd tension determinations were made using the American Curd-O-Meter and in a few cases the Submarine Signal Co. Curd Tension Meter (corrected) employing the procedure recommended by the American Dairy Science Association Committee on Methods of Determining the Curd Tension of Milk, in 1938.

In order to determine the degree of coagulation obtained in the superheating process more accurately than by visual means, reconstituted samples were centrifuged at 2500 r.p.m. for five minutes and the per cent of sedimentation measured. This made it possible to evaluate the effect of the degree of coagulation on reduction of curd tension and on digestibility.

Peptic digestibility was measured using the procedure employed by Doan and Flora<sup>1</sup> with some modifications, the results being based on the percentage of total nitrogen which passed a 10-mesh screen after three hours of digestion employing a progressive lowering of the pH to approximately pH 3.5 at two and one-half hours.

#### EXPERIMENTAL RESULTS

##### *Reduction of Curd Tension*

The average reduction in the curd tension value of superheated condensed, reconstituted and homogenized milk, based on four different trials, is shown in table 1, as are also similar data for samples of milk taken at different stages of the manufacturing process and used as controls for the sake of furnishing comparative evidence of the effects of the various treatments employed.

The results indicate that superheated condensed and reconstituted milk can be produced with a curd tension of zero (sample 8) without difficulty. However, homogenization of the superheated product either before or after reconstitution to the fluid state is a necessary practice since in most cases the unhomogenized product contained fine flakes of protein. After homogeniz-

<sup>1</sup> Doan, F. J., and Flora, C. C. Comparative Digestibility of Some Soft Curd Milks in Vitro. Bul. 380, The Pa. Agr. Expt. Sta. 1939.

ing the texture is absolutely smooth and indistinguishable from ordinary homogenized fluid milk. As indicated by samples 8, 9 and 10 in table 1,

TABLE 1

*The effects of condensing, superheating, reconstituting and homogenizing on the curd tension value of fluid whole milk*

(Average data obtained in four trials where the superheating temperature varied from 194° F. to 205° F. and the time varied from 10 to 30 minutes)

Treatment of milk	Curd tension value
	<i>grams</i>
1. Preheated at 145° F. for 30 minutes (fluid)	37.6
2. Preheated and homogenized (fluid)	14.2
3. Preheated and superheated (fluid)	2.8
4. Preheated, superheated and homogenized (fluid)	4.2
5. Preheated, condensed, and reconstituted	28.2
6. Preheated, condensed, reconstituted and homogenized	17.0
7. Preheated, condensed, homogenized and reconstituted	20.2
8. Preheated, condensed, superheated and reconstituted	0.0
9. Preheated, condensed, superheated, reconstituted and homogenized	0.25
10. Preheated, condensed, superheated, homogenized and reconstituted	0.25

homogenization has a tendency to increase the curd tension of the superheated product and the same effect is noticeable for the fluid milk treated at superheating temperatures (samples 3 and 4). In only one trial of the four, however, did samples 9 and 10 give other than zero curd tension readings. In this trial the superheating process was not carried to a point resulting in definite protein flakes. Subsequent studies indicated that definite flaking is required to obtain zero curd tension values of the finished homogenized milk.

Table 1 indicates quite conclusively that the superheating treatment alone, or in conjunction with homogenization, will not reduce the curd tension value of fluid milk to zero (samples 3 and 4). The condensing process alone is considerably less effective in producing a milk of extreme soft curd character (sample 5). Condensing coupled with homogenization results in considerable reduction in curd tension (samples 6 and 7) but not to the extent that superheating alone reduces the value in the case of fluid milk (sample 3). From these data it is apparent that a partial coagulation of the milk is required to lower the curd tension to a zero value. For fluid milk this would undoubtedly require heating under pressure or boiling for some period of time but for concentrated milk it can be had in the more convenient temperature range of 180° F. to 205° F.

### *Digestibility*

Digestion analyses were made on all of the samples collected in the four trials mentioned previously. The results are presented in table 2, the values given being the per cent of total nitrogen passing through a 10-mesh screen after 3 hours of digestion. There are a number of interesting deductions

TABLE 2

*The effects of condensing, superheating, reconstituting and homogenizing on the in-vitro digestibility of fluid whole milk*

Treatment of milk	Digestibility—% total N passing 10 mesh screen after 3 hrs. of digestion			
	Trial 1 C.T.—34.2*	Trial 2 C.T.—30.2*	Trial 3 C.T.—37.0*	Trial 4 C.T.—48.8*
1. Preheated at 145° F. for 30 minutes (fluid)	59.8	65.8	59.9	47.34
2. Preheated and homogenized (fluid)	65.7	78.6	68.9	47.13
3. Preheated and superheated (fluid)		79.0	83.4	62.39
4. Preheated, superheated and homogenized (fluid)		87.3	86.7	82.53
5. Preheated, condensed and reconstituted	61.3	59.3	58.9	49.7
6. Preheated, condensed, reconstituted and homogenized	65.8	64.6	64.1	57.48
7. Preheated, condensed, homogenized and reconstituted	61.5	62.6	62.9	52.15
8. Preheated, condensed, superheated and reconstituted	68.7	97.3	93.6	96.3
9. Preheated, condensed, superheated, reconstituted and homogenized	69.4	94.1	92.2	90.0
10. Preheated, condensed, superheated, homogenized and reconstituted	75.2	94.6	87.0	90.9

\* Curd tension value of the fluid milk after preheating:

Trial 1—Superheated at 194° F. to a heavy body but not protein flakes

Trial 2— “ “ 205° F. to a visible coagulation

Trial 3— “ “ 201° F. “ “ “

Trial 4— “ “ 205° F. “ “ “

which can be made from the data shown. Of prime importance in this study, however, are the high figures for digestibility of the reconstituted superheated condensed milk in trials 2, 3 and 4 (samples 8, 9 and 10). With one exception (sample 10, trial 3) these are all in excess of 90 per cent which indicates a high degree of digestibility comparable to that of reconstituted evaporated milk, eight samples of which had digestibility values ranging from 92.3 to 98.9 with an average of 95.3 per cent; and superior to ordinary boiled milk, eight samples of which had values ranging from 70.3 to 84.4 with an average of 76.8 per cent; but inferior to properly acidified milk and breast milk both of which exhibited values of practically 100 per cent.

It can therefore be assumed that properly made superheated soft curd milk with a curd tension value of zero would be as suitable for infant feeding purposes as is evaporated milk.

The data in table 2 indicate that the superheated samples of trial 1 had inferior digestibility characteristics to those of trials 2, 3, and 4. The explanation is that the milk in trial 1 was not superheated far enough to produce a definite flaking of the protein and the degree of precoagulation, therefore,

was insufficient to prevent considerable pepsin coagulation in the digestion determination. A definite pre-coagulation in superheating is indicated as necessary by the digestion data just as in the case of the curd tension data of table 1.

If the results presented in table 2 are compared with those shown in table 1 certain anomalies are to be noted in the relationship between curd tension and digestibility. Homogenizing fluid milk that has been heated to superheating temperatures increases curd tension values to some extent but definitely improves digestibility. On the other hand, homogenizing reconstituted superheated condensed milk has little or no effect on curd tension values but the digestibility, in these cases, is detrimentally affected. An exception to the latter statement is to be noted in the case of trial 1 which was not superheated enough to produce a product of high digestibility. Here homogenization, particularly when applied to the concentrated rather than the reconstituted milk, very appreciably improved the digestion characteristics but raised the curd tension from zero to 1 gram. This was the sample responsible for the average tension of 0.25 grams in samples 9 and 10 of table 1. The other three had zero values.

#### *Sedimentation*

It was noted that superheated condensed milk, after reconstitution, tended to separate, depending on the degree of superheating. In some cases a very noticeable sediment collected in the bottom of the container. Since a definite degree of flaking was found necessary to produce a highly digestible type of milk with a curd tension value of zero, it was believed that the sedimentation tendency might be made use of in controlling proper superheating. By centrifuging reconstituted samples various degrees of coagulation, produced by the superheating process, could be easily noted. Table 3 shows some preliminary data obtained in a study to determine the degree of coagulation required to insure a zero curd tension value after the homogenization process was applied.

It will be noted that, while all of the condensed, superheated and reconstituted samples exhibited zero curd tension values, only those coagulated to a point where 30 or 32 per cent sediment appeared on centrifuging showed zero values after homogenizing. It is believed that a superheating treatment sufficient to produce a 32 per cent sedimentation is the minimum heat treatment necessary to produce highly digestible milk. The percentages of sedimentation for sample 8 of the four trials included in table 2 were 20.0, 37.0, 32.0 and 35.0 respectively. Trial 1 showed poorest digestibility being no better than average boiled milk, even after homogenization which in the case of this trial improved the digestibility.

Notwithstanding the fact that homogenization somewhat lowers the digestibility of superheated milk it is a process necessary to smooth texture

TABLE 3  
*The effects of superheating to various degrees on the curd tension values and on the sedimentation of reconstituted, superheated milk before and after homogenization*

Treatment of milk	Curd tension (grams)						Sedimentation on centrifuging (%)					
	Trial						Trial					
	1	2	3	4	5	6	1	2	3	4	5	6
Condensed, superheated, and reconstituted	0.0	0.0	0.0	0.0	0.0	0.0	17.0	18.0	24.0	28.0	30.0	32.0
Condensed, superheated, reconstituted and homogenized	2.0	1.0	2.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5
Condensed, superheated, homogenized and reconstituted	2.0	1.5	2.2	1.0	1.0	0.0	0.0	0.5	0.5	0.5	1.0	1.0

and must be applied. There seems to be little choice in the case of properly coagulated milk whether this is accomplished while the milk is in concentrated form or after reconstitution as far as the digestibility results are concerned but, as can be noted in table 3, the sedimentation tendency is not overcome to quite as great a degree when the homogenization is applied to the concentrated milk as when it is accomplished after reconstitution. It seems, therefore, to be better practice to reconstitute the product first and then follow with homogenization.

### *Process*

In the preparation of a highly digestible superheated soft curd milk, the following process would appear to give consistently satisfactory results, from evidence collected in this study:

Select a high grade of milk of average composition. Preheat or forewarm this to such a temperature and hold for such a period of time as will result in satisfactory superheating conditions; or if pasteurization is required, satisfy the legal requirement for temperature and time. Condense the milk in a vacuum pan to a concentration of three to one. Superheat the product in the pan, or after withdrawing, at a temperature between 180° F. and 205° F. and hold until a definite flaking is evident. A sedimentation test of the reconstituted milk, such as is described under methods should give a reading of 32 to 35 per cent at this point. Immediately after the proper degree of coagulation is obtained the milk should be cooled under the coagulation point, to about 160° F. Water previously heated to 160° F. is then added in exact amount, on a weight basis, to bring the composition to the same level as in the original fluid milk. The product is then homogenized at 3000 pounds pressure followed by filling into the final container. A sedimentation test of the finished product should not give a reading of more than one per cent and the product should be absolutely smooth in texture with a noticeable cooked but not caramelized, flavor.

### SUMMARY AND CONCLUSIONS

A method of producing soft curd milk by heat treatment similar to that used in manufacturing superheated plain condensed milk is described. This type of soft curd milk has been designated as superheated soft curd milk.

Superheated soft curd milk properly processed, has a curd tension value of zero, is superior in digestion characteristics to boiled milk and comparable to evaporated milk, both of which have been long and successfully used as substitutes for breast milk. It is not, however, as digestible as acidified milk properly acidified to the isoelectric point.

It is believed that superheated soft curd milk has an advantage over acidified milk in that the flavor is not sour. Therefore, less difficulty would be experienced in substituting it in the case of infants started on breast milk.



The product, being equally as digestible as evaporated milk, could be used in place of the canned milk by those preferring a fresh milk product. Furthermore, superheated soft curd milk would provide the fluid milk dealer with a highly satisfactory type of soft curd milk (requiring no boiling in the home if carefully prepared) capable of competing with evaporated milk.

Since superheated soft curd milk contains no added foreign material, has had nothing removed and has not been treated in any way unusual to long established dairy plant practice, there should be less objection among health officials and pediatricians to this method of preparing a soft curd milk suitable for infant use than to some of the modifications previously advocated.

# REPEATABILITY OF TYPE RATINGS IN DAIRY CATTLE\*

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## INTRODUCTION

The usefulness of type for predicting production in dairy cattle has been investigated often. Although the correlations found between type and production have usually been small, type has persisted as a criterion in the selection of breeding animals. It appears that differences in type will continue to affect the price which buyers will pay for animals, or at least the ease with which different animals can be sold. Hence it is desirable to know something about the permanence, accuracy and other characteristics of estimates or ratings of type. The present study was undertaken to ascertain the amount and kind of variation occurring in type ratings when such ratings were made by different judges at intervals throughout an animal's life. The specific questions investigated were (1) the comparative accuracy of ratings made at different ages, (2) the repeatability of ratings separated by varying intervals of time, (3) the degree of agreement between judges in ratings given the same cow, (4) the specific causes of large changes in ratings, and (5) the extent to which future ratings can be predicted from one or more past ratings.

## SOURCE AND NATURE OF DATA

The data for this investigation were taken from the Holstein-Friesian herd at the Iowa State College during the period 1930 to 1940. Only the females were used in the present analyses, since few of the males were classified more than once. In all, 229 females were included, most of which were born in the college herd. The herd was fed and managed at a level sufficient to maintain good production. None of the animals was shown at fairs or expositions.

Two sets of independent ratings were made. One set was made primarily by the second author. A graduate assistant and the herdsman were usually present when these ratings were made and each of the three reached his own opinion before any of the others announced theirs. Then the junior author decided what the rating would be, sometimes shifting his original opinion as much as two points (two-thirds of an official grade) if the ratings of both of the other men were distinctly above or below his own. Thus, these ratings have something of the nature of an average. Scrupulous care was taken never to look at the record of any previous rating until the current one had

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been recorded. Each animal was rated during the weeks when it reached the ages of six months, 1, 2, 3, 4, 5, and 7 years. The terms and rating standards of the Holstein-Friesian Official Herd Classification were used as the standard except that the grades (excellent, very good, good plus, good, fair, and poor) were each subdivided into a high, medium and low. In this article this set of ratings is called "station ratings."

The other set of ratings, called "judges ratings" here, was made by nationally known dairy judges all but three of whom, at the time they made the ratings, were on the Official Herd Classification Committee of the Holstein-Friesian Association of America. The entire herd was classified at one time each year, usually during October or November. The 1930, 1932 and 1936 ratings were made by the same judge. A different man was used for each of the other years. The judges were asked to follow the principles and standards of Official Herd Classification except that they were to extend the classification to heifers as young as any on which they would venture an opinion. This usually included animals down to the ages of five or six months.

Considering only the cows that had freshened at least once, the official judges classified 2.2 per cent very good, 9.2 per cent good plus, 54.6 per cent good, 25.7 per cent fair, and 8.3 per cent poor. By contrast, the cows officially classified in the Holstein-Friesian voluntary plan up to Dec. 31, 1939, were distributed<sup>1</sup> as follows: 4.9 per cent excellent, 22.7 per cent very good, 24.0 per cent good plus, 41.2 per cent good, 6.6 per cent fair, and .6 per cent poor. These figures indicate that this herd was poorer in type than most of those classified voluntarily, but the real difference is doubtless less than the figures imply. In the voluntary system it seems inevitable that many of the owners would select a time at which their herds would show to best advantage, would give some special care and preparation to the animals prior to classification, and would eliminate some of the poorer individuals in type if such animals were not greatly needed in the breeding herd. In the herd which was the subject of this investigation there was little opportunity to cull for type during the period studied. The recorded causes and numbers of cows and heifers leaving the herd after the age of six months were: Bang reactors, 31; other breeding troubles, 24; tuberculosis reactors, 18; death, 13; mastitis, 12; low production, 10; dairy purposes, 3; poor type, 1; and miscellaneous, 13. However, the decision to remove cows for mastitis, low production, and some miscellaneous causes may have been influenced in part by their type.

For computation, the six grades of the judges' ratings were coded: poor, 1; fair, 4; good, 7; good plus, 10; very good, 13; and excellent, 16. The same code was used for the station ratings with the intervening numbers being used for the subdivisions of the classes.

<sup>1</sup> Norton, H. W. Extracts from A. R. Reports. *Holstein-Friesian World* 37: 618. 1940.

## RESULTS

*Variation in the Rating Levels of the Different Judges*

Table 1 shows the annual averages of the station and judges' ratings. The station ratings tended to increase over the period studied. There ap-

TABLE 1  
*Annual averages and standard deviations of the ratings*

Year	Station ratings		Judges' ratings	
	Mean	Standard deviation	Mean	Standard deviation
1930			5.9*	2.94
1931	6.8	1.82		
1932	6.9	2.56	6.0*	2.41
1933	7.2	2.49	7.6*	2.00
1934	7.5	2.81		
1935	8.3	3.26	7.8	3.84
1936	8.9	2.70	6.8*	3.21
1937	8.8	3.00	5.7*	2.58
1938	8.6	3.16	9.5	3.40
1939	9.0	2.62	6.8*	1.87
1940	9.0	2.46	6.7	2.91

\* These ratings were made by judges on the official herd classification committee.

peared, however, to be no objective method of determining whether this increase was due to improvement in the herd, a change in the ideals of those classifying the cows, or both. In the judges' ratings no regular trend existed. There was, however, considerable variation between the annual averages, there being approximately 4 points or  $1\frac{1}{2}$  official grades between the high and low averages. If only those who were on the official classification committee are included, the range between their averages was 1.9 points or about two-thirds of an official grade. Again there was no objective method for analyzing the causes of this variation. The lack of any consistent trend here, however, would seem to indicate that much of the variation was due to differences in the rating levels which the various judges used. That those who were on the official committee differed less in their averages than the others seems to indicate, as might be expected, that the meetings held to unify and standardize the official terms had some influence in the desired direction. However, the difference between consecutive judges' ratings, when omitting men not on the official classification committee, was statistically significant.<sup>2</sup> This, of course, could mean either that the judges levels genuinely differed, or that the average type of the herd had really changed, or both. Naturally about a fifth (or a little more) of the animals

<sup>2</sup> In this article the word "significant" has been used to indicate that such a value would occur by chance in not more than five per cent or less than one per cent of the trials when no such difference existed in the population from which these data are considered a random sample. "Highly significant" has been used to indicate that a value that large or larger would occur by chance in less than one per cent of the trials.

classified one year would be gone from the herd before the next classification and would be replaced by nearly an equal number of heifers.

To eliminate the variation in the rating levels of different judges, all ratings were adjusted to a common level in those analyses subsequent to table 1 wherever judge-to-judge differences in general rating levels could have affected the findings.

### *Comparative Accuracy of Ratings at Different Ages*

Table 2 shows for both sets of ratings the correlation coefficients between ratings at each pair of ages. The coefficients varied considerably

TABLE 2  
*Correlations of all age ratings*

Station ratings			Judges' ratings		
Ages correlated	D/f	r	Ages correlated	D/f	r
½ yr. with 1 yr.	133	.43++	1st with 2nd	58	.21
1 " " 2 "	111	.49++	2nd " 3rd	76	.27+
2 " " 3 "	93	.49++	3rd " 4th	56	.30+
3 " " 4 "	64	.55++	4th " 5th	56	.48++
4 " " 5 "	42	.83++	5th " 6th	27	.40+
5 " " 7 "	13	.61+	6th " 7th	10	.37
			7th " 8th	3	-.28
All consecutive ratings		.52	All consecutive ratings		.32
½ yr. with 2 yrs.	103	.16	1st with 3rd	40	-.03
1 " " 3 "	79	.44++	2nd " 4th	67	.22
2 " " 4 "	61	.48++	3rd " 5th	21	.16
3 " " 5 "	37	.66++	4th " 6th	27	.49++
4 " " 7 "	10	.36	5th " 7th	10	.43
			6th " 8th	3	-.23
All with two rating interval		.37	All with two year interval		.20
½ yr. with 3 yrs.	71	.34++	1st with 4th	16	.00
1 " " 4 "	51	.33+	2nd " 5th	31	.34+
2 " " 5 "	32	.56++	3rd " 6th	5	.56
3 " " 7 "	8	.22	4th " 7th	10	.36
			5th " 8th	3	-.22
All with three rating interval		.37	All with three-year interval		.26
½ yr. with 4 yrs.	45	.21	1st with 5th	4	.59
1 " " 5 "	25	.44+	2nd " 6th	11	.65+
2 " " 7 "	4	-.45	4th " 8th	3	-.46
			2nd " 7th	6	.62
			2nd " 8th	2	-.88
All with four-rating interval		.24	All with four-year interval		.46
½ yr. with 5 yrs.	21	.01			
1 " " 7 "	2	.30			
All with five-rating interval		.01			

+ indicates a significant correlation.

++ indicates a highly significant correlation.

but much of this is presumably due to the small number of animals in several of these comparisons.

The coefficients between consecutive age ratings increased slightly and rather steadily with age up to four or five years and then decreased. Although none of the consecutive differences were large or statistically significant in these data, they suggest that type ratings are slightly more repeatable near maturity than at very young or very old ages. Contrary to our expectation there was no noticeable increase in repeatability following a heifer's first freshening.

In table 3 the correlation coefficients have been averaged to show the relation of each age rating with all others. The first ratings were distinctly the

TABLE 3  
*Correlations of each age rating with all other ratings*

Station ratings			Judges' ratings		
Ages correlated	Pairs compared*	r	Ages correlated	Pairs compared	r
1 yr. with all others	385	.28	1st with all others	126	.11
1 " " " "	413	.44	2nd " " " "	265	.26
2 " " " "	416	.40	3rd " " " "	208	.27
3 " " " "	364	.47	4th " " " "	249	.31
4 " " " "	285	.48	5th " " " "	166	.38
5 " " " "	182	.57	6th " " " "	95	.42
7 " " " "	49	.33	7th " " " "	49	.36

\* Each age rating was used several times in comparing it with all others, thus the number of degrees of freedom is somewhat less than the pairs compared. Since it is not readily apparent how many degrees of freedom should be deducted because of this repetition, the number of pairs of ratings compared has been given in place of degrees of freedom.

least like the other ratings. For all ratings after the first the coefficients were of approximately the same magnitude but there was some slight (statistically non-significant) increase up to four and five years of age. Apparently type can be judged on heifers as young as one year almost as accurately as on mature cows. Both the station and judges' ratings showed this trend.

TABLE 4  
*Correlation of each age rating with all others when omitting six-month station ratings and judges' first ratings*

Station ratings			Judges' ratings		
Ages correlated	Pairs compared	r	Ages correlated	Pairs compared	r
1 yr. with all others	278	.44	2nd with all others	205	.28
2 " " " "	311	.48	3rd " " " "	166	.29
3 " " " "	291	.50	4th " " " "	231	.33
4 " " " "	238	.53	5th " " " "	160	.37
5 " " " "	159	.65	6th " " " "	95	.42
7 " " " "	47	.35	7th " " " "	49	.36

If the lower correlation coefficients involving the oldest ages need an explanation, perhaps it can be found in the fact that some cows are then beginning to show broken udders and other age effects that affect type ratings seriously.

Table 4 shows the correlation of each age rating with all others when the first ratings were omitted. This increased the size of the coefficients but in no way changed the relationships just discussed.

#### *Station Ratings Compared with Judges' Ratings*

It is noticeable in this study that the station ratings were more repeatable than the judges' ratings. Although the differences between the two classifications did not reach the level of statistical significance at most ages, they were consistently in favor of the station ratings. The reasons for this are not certain. Unquestionably the judges had far more experience in judging and had studied type more intensively than the station men. Factors that might have operated to make the station ratings more repeatable than the judges ratings are: (1) the station ratings were somewhat of an average of three independent opinions, (2) the judges were different men ~~most~~ years and may thus have differed more in their ideals than the station men, some of whom were the same throughout the study, (3) the station men may have been influenced by past knowledge of the animals although every precaution was taken to avoid looking at or remembering previous ratings, and (4) the classes in the station ratings were subdivided into high, medium, and low which would permit greater accuracy of classification. The fourth explanation seems not to have been important when tested by Sheppherd's correction. Of the remaining three we incline to think that the first cause is the most important with the second coming next and the third of practically no consequence, but we can find no objective way of verifying this belief.

#### *Effect of Length of Interval*

Table 2 includes separately the averages of the correlations between ratings separated by one, two, three, four and five rating intervals. In the station ratings the size of the correlations decreased with an increase in the time interval. In the judges' ratings no such general trend was observed. Since the ratings made at the youngest age were somewhat less repeatable than the others the averages of the correlations were computed after omitting all of the first ratings. The correlation coefficients for the station ratings under these conditions were .55++, .51++, .40++, and .38++ when separated by intervals of one, two, three, and four years, respectively. The corresponding averages for the judges' ratings were .34++, .26++, .34++ and .45, respectively. Thus, consecutive ratings when made at or above one year of age, were little if any more repeatable than non-consecutive ones. Apparently most of the things which cause changes in the type ratings of an animal operate over a relatively short period, so that their incidence from one year to the next is

almost random. Ratings of the same animal made only one year apart differ almost as much as ratings separated by two or more years.

### *Agreement between Different Judges*

Correlation coefficients were calculated for each pair of judges between the ratings they made on the same cows in different years. This was done to test whether certain judges agreed with each other more than others or whether the variation was no more than might reasonably occur when sampling from one population. The first age ratings were included in these comparisons as otherwise the numbers of animals involved would have been very small. Table 5 is a nearly typical one of these correlation tables. The coefficients for all of them are shown in table 6. The herd was not classified by judges in 1931 or in 1934. The same judge made the 1930, 1932, and 1936 ratings.

TABLE 5

*Sample correlation table between ratings which two official judges made on the same cows*

1936 judge	1937 judge			
	Poor	Fair	Good	Good plus
Very good		(1)	(3)	
Good plus	(1)	2 (2)	1 (3)	
Good	1	(2)	4 (5)	1 (1)
Fair	4 (2)	2 (3)	1 (1)	
Poor	1 (1)	(2)	(2)	

The numbers in parentheses were individuals that had not yet freshened when the 1936 rating was made. A few of these had still not freshened in 1937. Numbers not in parentheses were cows which had already freshened in 1936 and for which both judges could therefore see how the udder looked after freshening.  $r = +.37$  for the whole group, which is a shade higher than the average of the judges' ratings.

The averages involving different judges (correlations weighted and averaged by the z-method of Fisher) were:

1930, '32, and '36 judge with himself	.56
1930, '32, and '36 judge with others	.31
1933 judge with others	.27
1935 judge with others	.30
1937 judge with others	.26
1938 judge with others	.25
1939 judge with others	.38
1940 judge with others	.21

With the exception of the one judge with himself, the correlation coefficients varied no more than if all of them had been random samples from one population. Attempts to pick groups of two or more judges who agreed among themselves but not with the others failed or were inconclusive. Thus, the 1935 and 1936 judges agreed more than average with each other and with the 1932 judge but the three of them were not consistent in disagreeing with



TABLE 6  
Correlations between ratings by different judges\*

Ratings correlated	D/f	r
1932-1933	19	.38
1935-1936	46	.46++
1936-1937	45	.37++
1937-1938	74	.24++
1938-1939	78	.39++
1939-1940	88	.24++
All consecutive ratings		.32
1930-1932	18	.57++
1933-1935	42	.16
1935-1937	22	.14
1936-1938	35	.12
1937-1939	58	.21
1938-1940	63	.18
All with 2 year intervals		.18
1930-1933	15	.36
1932-1935	10	.61+
1933-1936	19	.23
1935-1938	15	.13
1936-1939	25	.23
1937-1940	45	.28
All with 3 year intervals		.26
1932-1936	2	.46
1933-1937	4	.63
1935-1939	12	.27
1936-1940	19	-.04
All with 4 year intervals		.12

\* The 1935, 1938, and 1940 judges were not on the Holstein-Friesian Official Classification Committee.

the others. The 1933 judge agreed well with both the 1930 and the 1932 ratings but less than the average amount with the 1936 ratings although all three of these were by the same man. These comparisons seem to indicate that the judges could not be separated into groups within each of which the ideals were similar but distinctly different from those held in other groups. If the judges did differ genuinely in ideals, each apparently had his own peculiarities. The judges not on the official classification committee agreed with each other and with those on the official committee about as closely as the latter agreed with each other. As previously noted in table 1, however, these judges varied more in rating levels than did the members of the committee. This suggests that conferences and practice, such as are held in official classification work, help to unify judges in the general level of their rating standards. These conferences probably do little to promote a higher degree of agreement about the relative merits of different individual cows. At least this was true in these data where all of the judges had considerable judging experience.

It would be interesting to know how far the imperfectness of agreement concerning individual animals was due; (1) to genuine and permanent differences in ideals from judge to judge, (2) to the animal really changing in

appearance from one year to another, (3) to temporary fluctuation in the judge's ideal, and (4) to the unsuitability or clumsiness of this classification system as a means of recording exactly what the judge thought of the animal. The experimental design did not permit separating these different causes of disagreement. We believe that (2) was the most important and (1) the least important of these causes but this opinion is based on considerations not absolutely conclusive in themselves. These include such things as experience in estimating rate of gain and final values in beef steers,<sup>3</sup> some unpublished analyses of scoring technique as applied to swine, and some general considerations such as personal knowledge of individual cases where cows changed widely in general appearance within the space of one or two years.

### *Causes of Major Shifts in Type Ratings*

In the station classification brief descriptions of the animals were recorded each time a rating was made. In making these descriptions care was taken not to look at the previous records until after the current description had been written. A summary of the 132 animals that had three or more ratings showed that 42 changed one official grade or less, 64 changed two or less official grades but more than one, and 26 changed more than two grades. The causes of the larger changes were sought by examining the verbal descriptions. Among the cows that changed more than two official grades during the test period, it appeared that 50 per cent of these large shifts were caused mainly by udder changes, 26 per cent by obvious changes in general health, 12 per cent by inferior body conformation which later improved, and 12 per cent by miscellaneous causes. The larger shifts occurred frequently between consecutive ratings and apparently as often in the older animals as in the younger ones.

### *Value of Knowing More Than One Rating*

The correlation coefficient for consecutive ratings was compared with multiple correlation coefficients in which the third rating was dependent on the first and second ratings, and the fourth rating was dependent on the first, second, and third ratings. The first age ratings in both classifications (those made at less than ten months of age) were omitted in these correlations. In the station classification 61 animals had four or more ratings. The correlation coefficient of the third and fourth ratings was .62; of the fourth as dependent on the second and third ratings, .65 -; and of the fourth as dependent on the first, second, and third ratings, .65 +. In the judges' classification 33 cows had four or more ratings. The correlation coefficient of the third and fourth ratings was .10; of the fourth as dependent on the second

<sup>3</sup> Partly published in Jour. Agr. Res. 42: 853-881, and partly unpublished studies at the Iowa Station.

and third ratings, .50; and of the fourth as dependent on the first, second, and third ratings, .57. Thus, little was gained in the station ratings by using the second preceding rating while much was gained in the judges' ratings. That the advantage was small in the former classification was probably due to the fact that the correlation coefficient between the third and fourth ratings was unusually high. When the first age ratings were omitted the correlation coefficients for all consecutive ratings was .55 in the station classification and .34 in the judges' classification. In data with such correlations one would generally expect to get larger gains by the use of two or more ratings than was found in the station classification and smaller gains than was found in the judges' classification. If the correlations between the three ratings all equalled .55, one would expect to make about 1.14<sup>4</sup> times as much improvement in the third rating by selecting on the average of the first two ratings as by selecting on either one alone. If all the correlations equalled .34, the corresponding figure would be 1.22. Although there were not enough animals in either comparison to determine accurately the value of using more than one rating, the use of two ratings appears desirable whenever possible. The use of three or more ratings, however, would further increase the gains only a little even if all the correlations were equal, and perhaps not enough to be worth attention if correlations between consecutive ratings really are a little higher than those between ratings separated by longer intervals of time.

#### DISCUSSION

The small correlation of the first ratings (those made at 10 months of age or less) with all future ratings shows that little confidence can be placed in ratings made under one year of age. At or above one year of age the repeatability of the ratings was somewhat higher and of approximately the same magnitude at all ages. Thus, estimates of type at any age above one year are about equally valid for predicting a cow's future type.

The repeatability of type, .55 in the station ratings and .34 in the judges' ratings, is of the same order as the repeatability of intra-herd production records. Intra-herd correlation coefficients between yearly or lactation fat records made by the same cow have been in most studies about .4, rarely being below .3 or above .5. The repeatability of milk records appears to be about as high, although milk records have not been studied for this as much as fat records. Thus, one type rating made at or above one year of age is about as indicative of a cow's future type as one production record is of her future production records. In view of this it appears that breeders should be conservative in culling or selecting for type on the basis of a single inspection. The animal may appear much better or worse next year.

<sup>4</sup> This equals  $\sqrt{\frac{2}{1+r}}$ .

If animals do genuinely change in type from time to time as much as we think, a person wishing to measure type or select for it will gain considerably by having each animal rated more than once during its life. More is to be gained by that than by striving to get some one particular judge to do the rating. If, as these data indicate, about 30 per cent of the variance in single type ratings made by one judge is due to things which are permanent for those individual cows and will be agreed to by subsequent judges, then in comparable populations, in which each cow had been rated in two different years, about 46 per cent ( $= \frac{2r}{1+r}$ ) of the variance in the averages for individual cows would be due to genuine and permanent differences between the cows. If each cow had three ratings, the corresponding figure would be 56 per cent. If the average repeatability is higher than 30 per cent, the figures corresponding to these just given would be higher but would not increase so rapidly with additional ratings. One desiring seriously to breed for type and to use the services of judges not biased by personal knowledge of the production or preceding histories of the animals might well make it a regular practice to have his herd classified once every two years. If in addition the classification were extended to heifers as young as one year, and the present data indicate that the accuracy would not be lowered much thereby, then nearly all cows which freshen at all would be classified at least once, many of them twice, and some three or more times. The expense, the owner's opinion of how much he needs such help, and the advertising value of such a classification would need to be considered before adopting such a plan.

Proponents of type may wish to reinvestigate the old type-production problems to determine what changes in production might be accomplished by selecting for type when several type ratings and several production records are considered. If the lack of repeatability in type ratings is in large part due to random errors of classification, two or more ratings should show more relation to average future production than one.<sup>5</sup>

If type classification becomes more general, many more judges than are now being used to classify herds will be needed. This raises the questions: (1) Who is qualified to classify herds? (2) Are ratings made by different

<sup>5</sup> This was sketchily investigated in this herd with the following results:

Station ratings with six-month ratings omitted:	D/f	r
1 type rating with 1 production record	129	.25
2 type ratings with 2 production records	83	.45
3 type ratings with 3 production records	47	.26
Judges' ratings with 1st ratings omitted:		
1 type rating with 1 production record	143	.19
2 type ratings with 2 production records	89	.38
3 type ratings with 3 production records	49	.18

judges comparable? Although this experiment was not designed to answer these questions specifically it has thrown some light upon them. The agreement between judges in ratings given the same cows was of practically the same magnitude for both official and non-official judges, indicating little if any difference in the ideals of those rating the herd. (However, the non-official judges were men of wide experience in judging major shows.) It seems likely that special conferences can do little to unify experienced men in their relative rankings of the individual animals. In the average rating levels or standards there was considerable variation between judges. The conferences of the official judges may well have helped some in this respect. Within the official group, however, there was evidence of genuine differences in levels. How much of this could have been eliminated by more conferences among the judges is uncertain. Certainly if it becomes necessary for many men to classify herds, and if customers come to attach much importance to such ratings the judges should participate in such conferences and be given special schooling in rating standards, so far as that contributes much to making their rating levels alike. But it does not appear that any amount of such training will lead to near-unanimity of their ratings of individual animals, at least if those ratings are separated by a year or more in time.

#### SUMMARY

Type as shown by consecutive ratings was only a moderately permanent attribute of a dairy cow, being roughly similar in repeatability to her intra-herd production records. The repeatability of type when omitting ratings made at 10 months or less of age was .34 in the judges' ratings and .55 in the station ratings.

Ratings made under one year of age were somewhat less repeatable than those made at older ages. The increase in permanency of ratings after one year of age was small.

Consecutive ratings were only slightly if any more alike than ratings separated by two, three, and four years.

Some judges agreed with each other more closely than they did with other judges but these differences did not reach the level of significance here and seemed no more than would be expected from the fluctuations of random sampling.

Changes in the udder and in the health of the cow appeared to be the chief causes of large shifts in type ratings. Two ratings had an advantage over one for predicting future type but little if any more seemed to be gained from using more than two.

# SIZE OF THE RABBIT MAMMARY GLAND WITH SUCCESSIVE LACTATIONS\*†

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Milk and butterfat production of dairy cows have been shown to increase with advancing age up to about eight years. Based on official records this amounts to an increase of between 35 and 50 per cent of mature records over the two-year-old lactations (see Turner (6) for review). Under D. H. I. A. conditions the increase has been found to be 20 to 29 per cent in milk production (3, 1).

It is interesting to speculate as to the causes of this increase of production with age. The most obvious changes in dairy cattle with advancing age are an increase in body size and the recurrence of pregnancy and lactation. It would appear from studies which have been made of this subject (2, 5, 7) that from 40 to 80 per cent of the increase in production with age is due to recurring pregnancy and lactation and the development of organs and glands accompanying these conditions. Since size and quality of the udder have been shown to have a higher correlation with production than other body measurements (4) it appeared likely that much of this increase in production might be due to increasing development of the mammary glands with succeeding pregnancies. Measurement of the volume of the mammary gland in the living cow is impractical. The comparatively simple mammary glands of laboratory animals are spread over the ventral body wall in a fairly thin layer. This should render possible the measurement of changes in volume of the gland with successive lactations.

This study is a report of an attempt to demonstrate whether there is a lateral extension of the mammary glands with successive pregnancies in a laboratory animal, the rabbit. It was thought possible that lateral increase would give a good approximation of increase in volume of the mammary gland in the rabbit.

Virgin, sexually mature does of the New Zealand White breed were selected and bred. Soon after the first parturition, when the mammary glands were filled with milk, a line was tattooed on the lateral body wall from the anterior to the posterior leg through the shaved skin outlining the lateral extent of the mammary glands. A hand electric tattooing needle was used with India ink. The rabbits were immobilized on an operating board for the tattooing and later examinations.

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After weaning of the litters the does were rebred. Following the second and successive parturitions, the extent of the mammary glands was examined in relation to the tattoo line. Three does were carried through two, one through three and two through four parturitions. On ten examinations of the mammary glands in relation to the tattoo line there was no lateral extension apparent. In one case, after the second parturition, the mammary glands on the left side of the body extended uniformly one-quarter inch beyond the tattoo line.

These almost uniformly negative results do not preclude the possibility that the thickness and density of the mammary glands may have increased. That the anterior-posterior extent of the glands could have increased is doubtful, except for the first and last glands on a side, for the glands on the same side of the body are adjacent even at the first lactation. The glands also practically meet at the mid-ventral line.

#### SUMMARY

It was thought that the rabbit might illustrate the influence of mammary gland development in dairy cows as a cause of the increase in milk production with succeeding pregnancies. This increased production is greater than can be accounted for by the increase in body weight. The lateral extension of the mammary glands in succeeding lactations was compared in rabbits with lines tattooed in the skin at the lateral extent of the mammary glands early in the first lactation. In eleven succeeding lactations only one case was found in which the mammary glands on one side extended past the tattoo line. The lateral extent of the glands did not increase in the other cases.

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# FURTHER STUDIES ON THE USE OF SALT FOR IMPROVING THE QUALITY OF CREAM FOR BUTTERMAKING<sup>1</sup>

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Improvement of the quality of cream for buttermaking purposes by the addition of salt has received considerable attention during the past several years, although the method has not been accepted by either creamerymen or regulatory officials. The literature on the subject was reviewed in an earlier publication from this Station (1). In this earlier publication the addition to cream of 10 to 13 per cent salt, calculated on a fat-free serum basis, was shown to improve considerably the quality of the cream and the butter made therefrom under laboratory conditions simulating those on Kansas farms during the summer months (1). These studies did not answer a number of important questions, especially questions relative to the general applicability of this method to the handling of cream on the farm.

The studies herein reported were designed for the following purposes:

1. To study under controlled laboratory conditions the feasibility of adding at the beginning all of the salt necessary for the final volume of cream collected in daily increments.
2. To obtain information as to the applicability of the method to practical farm conditions, especially where all of the salt for a given quantity of cream was added to the container at the time collection was begun.
3. To follow the quantitative and qualitative changes in microflora under different holding conditions.
4. To obtain further data on the comparative scores of butters made from salted and unsalted creams.
5. To determine the effect of salt added to the cream on the containers used for collection and delivery.

## METHODS

In each of four series of laboratory trials, commercial salt was added in amounts, calculated on a fat-free serum basis, to give 10 or 13 per cent concentration in 2 liters of cream. In these series cream standardized to 30 per cent butterfat was used. Sterile gallon-size glass jugs were used in series I, II and III and single-service gallon tinned ice cream cans were used in series IV. In series I and II all of the salt and cream were mixed together on the first day and the resulting mixture held without addition for 10 days. In series III and IV the salt for the entire period was added to 200 ml. of cream

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the first day and 200 ml. of fresh cream was added with thorough stirring on each of the following nine days.

Four additional series of trials were conducted under farm conditions. In each case a portion of the cream from each separation was added to a control can, and another portion of the same cream was added to a can in which had been placed at the beginning of the collection period enough salt to give a final 13 per cent concentration in the fat-free serum of all cream added during the collection period. The cans of cream were agitated after each addition of cream, but the salt in cream VIIB was not completely incorporated due to lack of sufficient agitation during collection. In three of the four series of farm studies the cream (30-36 per cent butterfat) was cooled by immersion of the cans in a tank through which well water flowed. In the fourth series the cans of cream were allowed to stand at atmospheric temperature which averaged about 85° F. After the cans of cream reached the laboratory, samples for bacteriological and chemical analysis were taken and portions were then neutralized, when necessary, pasteurized at 149° F. for 30 minutes, cooled and churned in gallon glass churns. In each series butter from unsalted cream was salted to give as closely as possible the same salt concentration as was found in butter made from salted cream. No additional salt was added to the butter churned from the salted cream.

All grading of cream was done organoleptically by two or more judges working independently on samples designated by code. Cream grades as defined in the Kansas dairy law (2) were used as a basis for grading. Saltiness was not considered an undesirable flavor defect in samples to which salt had been added. Butter samples were graded in a similar manner, using generally accepted commercial grades as a basis.

Direct microscopic counts were made by the methods outlined in Standard Methods for the Examination of Dairy Products (3), except that dilutions of one part of cream in nine parts of water were prepared from those samples in which so many organisms were present that enumeration in an undiluted sample was difficult. Molds and yeasts were determined on acidified potato-dextrose agar by the methods outlined for butter in Standard Methods. Total plate count and acid formers were determined on lactose beef infusion agar to which 0.1 ml. of 0.16 per cent aqueous brom-cresol-purple per plate had been added. The plates were incubated at 20° C. and colonies of acid-formers were counted after 2 and 4 days. Total counts were obtained after 4 days. Counts of proteolytic and lipolytic bacteria were determined by the use of beef infusion agar to which 0.5 ml. of 3 per cent cottonseed oil emulsified in 0.5 per cent agar and 0.5 ml. sterile skim milk per plate were added. The proteolytic colonies were recognized by the surrounding cleared areas in the somewhat opaque medium and lipolytic colonies by the blue color of the fat globules around the colonies after the plates had been flooded for several minutes with 0.1 per cent aqueous Nile

blue sulfate. In many instances counts of proteolytic and lipolytic organisms undoubtedly were inaccurate due to overgrowth by the many other organisms present.

Titratable acidity and formol titration were determined by the usual laboratory procedures.

The effect of salt upon metals was determined by immersion to a depth of 2 inches of polished strips of dairy metal, tinned copper and two kinds of stainless steel in cream containing 13 per cent salt (serum basis). Temperatures of 90° F. for 10 days and 150° F. for 18 hours were used, the temperature in the latter series being maintained for 1, 8, 6, and 3 hours, respectively, on each of four successive days. Corrosion was measured by visual observation and loss of weight by the metal strips as a result of immersion.

#### EXPERIMENTAL RESULTS

*Laboratory studies on the effect on cream and butter quality of the addition of salt to cream.* Two series (I and II) of studies were made to show the effect of adding all the salt to the full volume of cream at one time, the mixture being held for ten days at 70 and 82° F. Two additional series (III and IV), which were otherwise identical except that different lots of cream were used, were conducted to show the effect of adding all of the salt at the beginning and adding the cream in daily increments. The grade and chemical data on these four series are shown in table 1. The data indicate that the addition of salt markedly improves the quality of cream and the butter made therefrom, either when the salt and cream are all mixed at the beginning and held, or when all of the salt is added at first and the cream is added in daily increments. All of the cream to which salt was added remained sweet for four days and none of it had dropped to second grade after ten days. None of the butter made from salted cream held for ten days at 70 or 82° F. before churning graded below 90 when churned, and much of it graded 91 or 92. These results are in marked contrast to those obtained on the control lots. Much of the control cream was third grade (illegal) after ten days and the butter made from this cream graded from 86 to 89 immediately after churning. The data on acidity and formol titration emphasize the degree to which the salt reduces chemical changes caused by microorganisms while the cream is being accumulated on the farm. Increases in acidity and formol titration in the salted cream were small, especially in the two series in which all the salt was added at first and cream accumulated from day to day. This undoubtedly was due to the very high salt concentration during the first few days. This greater preservative action of the initially higher concentration of salt is not reflected in significantly better grades of cream and higher scores of butter. At the same salt concentration the differences in grade are smaller at 70° F. than at 82° F. Individual lots of cream seem to differ in the degree of improvement resulting from salt

TABLE 1  
*Laboratory studies on the effect of salt on the quality of cream and the butter made therefrom*

Series No.	Storage temp. (°F.)	Cream data												Butter scores					
		Acidity (per cent)			Formol titration			Cream grades						Fresh			After 60 days at 0° F.		
								After 10 days											
		0 days		10 days		After 4 days		After 10 days											
		0	0	10	13	0	0	10	13	0	10	13	0	10	13				
All salt added at start, cream accumulated daily (VI in glass container, VII in tinned container)																			
I	70	0.11	1.35	0.21	0.155	2.25	4.62	2.65	2.60	1	Sw*	Sw	3	1	1-	89	90		
II	70	0.11	0.77	0.125	0.12	1.80	3.05	1.75	1.70	1	Sw	Sw	2-	Sw	Sw	87	91.5		
I	82	0.11	1.96	0.29	0.21	2.30	6.05	2.75	2.70	2	Sw	Sw	3	1-	1-	89	90		
II	82	0.11	1.60	0.19	0.12	1.80	4.30	2.10	1.80	2-	Sw	Sw	3	Sw	Sw	86	92		
III	70		0.69	0.12	0.125		2.25	1.70	1.60	1	Sw	Sw	2-	1	1	88	92		
IV	70		0.68	0.105	0.11		2.40	1.80	1.60	1	Sw	Sw	2-	Sw	Sw	87	91.5		
III	82		1.60	0.145	0.135		3.10	1.85	1.70	1-	Sw	Sw	3	1-	1	86	91		
IV	82		1.63	0.115	0.12		3.70	1.85	1.65	1-	Sw	Sw	3	Sw	Sw	86	91.5		

\* Sweet.

addition, better creams and butters being usual in series II than in series I and series IV being somewhat superior to series III in this respect. The extremely low scores after storage for the butters from unsalted creams of series IV were due to a pronounced cheesy and putrid flavor apparently the result of bacterial action rather than to any change which might be attributed to the use of metal containers.

The data relative to total plate count and counts of acid-formers are presented in table 2. Total bacterial counts on series I and II show that when salt in the concentrations used was added to the cream, the count usually decreased somewhat on the first day and then increased to the end of the ten-day period. The highest counts reached by the salted creams were appreciably below the maximum levels reached in the unsalted creams. The superior quality of the original cream and the lower maximum counts of the salted creams explain why salted creams and the butters made therefrom were better in series II than in series I. In series III and IV, where the cream was accumulated over ten-day periods, both the magnitude and duration of the decrease in count resulting from the addition of salt were greater than in series I and II when all the cream and salt was added at the beginning, and the levels reached after 10 days tended to be slightly lower although of the same general magnitude.

In series I and II the acid forming organisms constituted almost 100 per cent of the total viable populations of the unsalted control creams until the total counts had declined considerably below the maxima, after which the count of acid-formers dropped more rapidly than did the total count. The daily addition of fresh unsalted cream to series III and IV controls tended to maintain a predominantly acid-forming bacterial flora at 70° F., but at 82° F. the proportion of acid-forming bacteria decreased significantly toward the end of the ten-day accumulation period. In salted creams the count of acid-forming bacteria usually dropped below the total count almost at once and acid-forming bacteria made up only a small proportion of the total count at the end of the ten-day period. This suppression of acid-forming bacteria explains why cream to which salt has been added seldom develops a high degree of acidity.

The direct microscopic counts given in table 3 also indicate the marked inhibitory action of salt upon bacterial growth but emphasize the fact that bacteria do grow extensively at the salt concentrations used. The counts at 10 days are not the maximum counts obtained in all instances, even on the salted samples, but in no series did a pronounced drop in microscopic count parallel that observed for the plate counts. In the unsalted creams the organisms observed were usually predominantly large paired and clumped cocci, probably micrococci, and occasional sarcina types were encountered. These are organisms which usually cause comparatively little change in the substratum in which they develop.

TABLE 2  
*Changes in plate counts of total and acid-forming organisms under laboratory conditions*

Series	Age days	Total count (thousands) on sample:				Acid-formers (thousands) on sample:							
		70° Control	70°-10%	70°-13%	82° Control	82°-10%	82°-13%	70° Control	70°-10%	70°-13%	82° Control	82°-10%	82°-13%
I	0	45,000											
	1	150,000	37,000	34,000	47,000	37,000							
	2	960,000	54,000	45,000	64,000	50,000							
	4	540,000	83,000	49,000	120,000	87,000	540,000						
	6	91,000	190,000	60,000	250,000	100,000	90,000						
	8	29,000	290,000	81,000	30	150,000	16,000						
II	10	9,900	230,000	101,000	750	170,000	Molds						
	0	16					16						
	1	550,000	6	8	1,100,000	230	9						
	2	1,300,000	120	11	1,100,000	6,500	58						
	4	1,300,000	1,000	700	1,100,000	28,000	1,300,000						
	6	920,000	26,000	2,800	9,200	93,000	920,000						
III	8	850,000	53,000	6,000	300	16,000	840,000						
	10	370,000	81,000	18,000	110	36,000	80,000						
	0	3,000											
	2	1,500,000	21	18	1,200,000	17	15	1,500,000					
	4	820,000	33	36	500,000	43	18	820,000					
	7	580,000	3,600	310	330,000	6,000	570,000						
IV	10	340,000	54,000	18,000	650	14,000	340,000						
	0	73					49						
	2	1,100,000	1,300	1,700	1,000,000	1,500	1,100,000						
	4	830,000	690	500	610,000	340	220	830,000					
	7	340,000	2,700	1,000	13,000	1,500	220,000						
	10	470,000	53,000	26,000	1,300	90,000	450,000						

TABLE 3  
Microbiological data on laboratory studies of the effect of salt on the quality of cream (samples incubated 10 days)

Series	Salt %	Temp.	Plate count per ml. of cream				Total plate count per ml. after past. 149°-30 min.	Direct microscopic count per ml.
			Proteolytic	Lipolytic	Yeasts	Molds		
I†	0	70	*	*	12,000,000	>1,000,000	2,500	948,000,000
	10	70	29,000,000	10,000,000	20	110	34,000	260,000,000
	13	70	15,000,000	2,000,000	140	150	28,000	140,000,000
	0	82	*	*	4,200,000	>100,000	1,100	936,000,000
	10	82	20,000,000	10,000,000	80	<10	34,000	264,000,000
II‡	13	82	19,000,000	5,000,000	250	<10	46,000	372,000,000
	0	70	200,000	<1,000,000	<100,000	700,000		1,240,000,000
	10	70	3,500,000	200,000	1	2		205,000,000
	13	70	150,000	200,000	0	3		72,000,000
	0	82	*	*	1,000,000	11,000,000		1,385,000,000
III†	10	82	9,000,000	6,500,000	0	0		420,000,000
	13	82	1,000,000	700,000	0	3		141,000,000
	0	70	180,000,000	<1,000,000	1,300,000	<100,000	11,600	606,000,000
	10	70	<100,000	300,000	<10	150	9,700	88,000,000
	13	70	100,000	100,000	<10	150	10,000	36,000,000
IV†	0	82	<100,000	<100,000	450,000	<100,000	150	1,400,000,000
	10	82	4,500,000	<100,000	<10	100	8,800	42,000,000
	13	82	50,000	100,000	<10	140	10,400	47,000,000
	0	70	<1,000,000	<1,000,000	2,900,000	450,000	55,000	1,060,000,000
	10	70	200,000	<100,000	<10	20	56,000	86,000,000
	13	70	<100,000	<100,000	10	60	68,000	82,000,000
	0	82	<10,000	<100,000	1,400,000	280,000	650	624,000,000
	10	82	100,000	300,000	<10	20	71,000	92,000,000
	13	82	150,000	100,000	<10	20	68,000	108,000,000

\* Molds made proper plates uncountable.

† All salt added at once, cream added over 10-day period.

‡ All salt and cream added at once and held 10 days.

Data on the numbers of microorganisms of certain types developing in the different lots of cream are given in table 2, only values at the end of the 10-day period being recorded. Salt does not entirely prevent the development of proteolytic and lipolytic organisms in cream, but it is effective in preventing the development of molds and yeasts. Plate counts after pasteurization indicate that when cream was held at 70° F., the pasteurization efficiency was the same on salted cream as on unsalted cream. When cream was held at 82° F. the pasteurization efficiency on the unsalted cream was much higher than on the same cream held at 70° F., but no similar increase in efficiency occurred in the case of the creams to which salt was added.

The data definitely indicate that the addition of salt to cream is effective because of both quantitative and qualitative changes in the microflora which result.

*Farm studies.* Four series of studies of the effect of salt on the quality of cream and butter made therefrom were conducted on farms through the cooperation of two local dairy farmers. The results of these series are presented in tables 4 and 5. In all instances the cream to which salt had been added or the butter made from such cream was superior to the cream and butter from the same source without added salt. The effect of salt was more apparent when higher cream storage temperatures were used. The salted creams had lower acidities, lower formol titration values, lower total plate counts, lower proportions of acid-forming bacteria, much lower plate counts of yeasts and molds, lower direct microscopic counts and were higher in grade than were the unsalted control creams. The butters made from the salted creams scored one to five points higher than butters from the same lots of cream unsalted and also frequently maintained their grade better in storage. The studies made under farm conditions thus corroborate the results obtained under laboratory conditions and indicate that the addition of salt to cream as a means of delivering higher quality cream might be applicable to practical farm conditions.

*Corrosive effect of salt in cream on metals used in dairy equipment.* A product known as dairy metal, a tinned copper and two types of stainless steel were used in these studies. The results of the study are presented in table 6. Both types of stainless steel seemed very resistant to corrosion by salt in cream, either at 90° or 150° F. The recorded losses in weight probably were within the limits of experimental error and no change in appearance occurred. Dairy metal showed definite susceptibility to corrosion under the test conditions, the strips decreasing an appreciable amount in weight and showing slight visible corrosion at the point of contact between the metal and the surface of the cream. Tinned copper was corroded quite badly by the salted cream, all strips losing appreciably in weight and showing considerable corrosion at the point of contact between metal and cream surface. The tinned cans used in one series showed no visible corrosion attributable

TABLE 4  
*Farm studies of the effect of salt on the quality of cream and butter made therefrom*

Series	Pa- tron	Temp. range (°F.)	Salted or un- salted	Cream data (7 d.)			Butter data									
				Grade	Acidity %	Formol titra- tion	Mois- ture content %	Salt content %	Scores							
									Fresh		7 days at 70°F.		30 days at 40°F.		60 days at 0°F.	
									Score	Differ- ence	Score	Differ- ence	Score	Differ- ence	Score	Differ- ence
V	A	58-62	Un S	1- Sw	0.52 0.11	2.40 1.75	15.5 15.4	1.3 0.7	88 91	3.0	86 90.5	4.5	87 90.5	3.5	87 92	5.0
	B	60-68	Un S	1 Sw	0.59 0.13	3.00 2.00	15.6 15.7	1.3 1.3	90 91.5	1.5	89 90	1.0	88 91	3.0	89.5 92	2.5
	A	58-62	Un S	1- Sw	0.51 0.20	2.32 2.00	15.6 14.6	0.8 0.7	89 91	2.0	87 90	3.0	89 92	3.0	88 92	4.0
	B	62-66	Un S	1- Sw	0.55 0.26	2.82 2.05	13.2 14.8	1.0 0.7	90 91	1.0	89 90.5	1.5	88 91.5	3.5	88 92	4.0
VII	A	80-92 (air)	Un S	3 Sw-	1.10 0.18	2.85 1.65	16.8 16.4	1.0 0.7	87 90	3.0	87 90	3.0	87 90	3.0	87 89	2.0
	B	80-92 (air)	Un S	3 Sw-	1.19 0.15	3.10 1.65	15.4 14.4	0.7 1.0	89 91	2.0	89 90	1.0	87 91	4.0	88 91	3.0
VIII	A	59-62	Un S	2 Sw-	0.61 0.11	2.90 2.00	16.0 17.0	1.4 0.9	86 91	5.0	86 90.5	4.5	86 91	5.0	86 90.5	4.5



TABLE 5  
*Microbiological data on farm studies of the effect of salt on the quality of cream (7-day accumulation period)*

Series	Patron	Temp. (°F.)	Salted or unsalted	Plate counts per ml.						Total direct microscopic
				Total	Acid-formers	Proteolytics	Lipolytics	Yeasts	Molds	
V	A	58-62	Un	440,000,000	> 400,000,000	< 10,000	80,000	11,000	8,000	680,000,000
	B	60-68	S	4,300,000	1,700,000	110,000	30,000	60	30	11,000,000
VI	A	58-62	Un	700,000,000	700,000,000	600,000	200,000	2,000	350,000	670,000,000
	B	62-66	S	15,000,000	2,500,000	260,000	160,000	< 10	70	53,000,000
VII	A	58-62	Un	440,000,000	> 400,000,000	100,000±	< 100,000	40,000	450,000	740,000,000
	B	62-66	S	10,800,000	5,400,000	130,000	10,000±	70	< 10	60,000,000
VIII	A	80-92 (air)	Un	660,000,000	650,000,000	2,000,000	< 1,000,000	< 10,000	200,000	780,000,000
	B	80-92 (air)	S	55,000,000	14,000,000	2,700,000	< 100,000	< 10	370	380,000,000
IX	A	59-62	Un	47,600,000	46,000,000	10,000	20,000	150,000	580,000	1,220,000,000
	B	59-62	S	18,000,000	2,700,000	40,000	20,000	> 10,000	< 10	7
X	A	80-92 (air)	Un	1,300,000	900,000	35,000	10,000	< 1,000	71,000	1,075,000,000
	B	80-92 (air)	S	11,400,000	600,000	400,000	350,000	> 500	> 50	38,500,000
XI	A	59-62	Un	306,000,000	300,000,000	200,000	< 100,000	3,200,000	500,000	1,455,000,000
	B	59-62	S	27,200,000	750,000	900,000	< 100,000	160	10	120,000,000

\* Salt poorly incorporated.

TABLE 6  
*Corrosive effect of salt in cream on certain dairy metals*

Metal used	Initial weight	Final weight	Loss in weight	Visual change
	Metal strips immersed to a depth of two inches in cream containing 13 per cent salt serum basis for 10 days at 90°F.			
	<i>grams</i>	<i>grams</i>	<i>grams</i>	
Stainless steel 2B	12.4064	12.4064	0.0000	No visible change
Stainless steel 2B	12.3879	12.3878	0.0001	No visible change
Stainless steel 4B	10.7157	10.7157	0.0000	No visible change
Stainless steel 4B	11.0255	11.0254	0.0001	No visible change
Dairy metal	14.2577	14.2560	0.0017	Slight corrosive effect at surface of cream
Dairy metal	14.1633	14.1620	0.0013	Slight corrosive effect at surface of cream
Tinned copper	19.0582	19.0522	0.0060	Visible corrosion near surface of cream
Tinned copper	18.9674	18.9620	0.0054	Visible corrosion near surface of cream
Metal strips immersed in salted cream as described above and cream held at 150°F. for total of 18 hours				
Stainless steel 2B	12.3175	12.3170	0.0005	No visible change
Stainless steel 4B	10.9085	10.9085	0.0000	No visible change
Dairy metal	13.2388	13.2376	0.0012	Slight corrosive effect at surface of cream
Tinned copper	19.1886	19.1851	0.0035	Slight corrosive effect at surface of cream

to the use of salt, since some corrosion was apparent in cans which had contained both salted and unsalted creams. Had the cans been used repeatedly or had the immersion tests with the metal strips been repeated a number of times on the same strips, greater susceptibility to corrosion might have been apparent. The results indicate that if salt were added to cream on the farm, containers of more resistant types than some of those commonly employed probably would be necessary. Use of plant equipment made of the more resistant metals also might be necessary in processing salted cream.

#### CONCLUSIONS

The data herewith presented confirm previously reported studies demonstrating that the addition of 10 to 13 per cent salt to cream markedly increases the quality of the cream and of the butter made therefrom.

Placing all of the salt in the container at the beginning of the accumulation period and adding the cream in daily increments with thorough stirring each time was found satisfactory in both laboratory and farm studies. Such a procedure would be applicable to practical farm conditions.

The added salt not only markedly reduces the numbers of microorganisms which develop in the cream but also affects the types. The number of acid-forming bacteria in the cream is reduced markedly, usually being less

at the end of the ten-day holding period than in the original cream. Salt in the concentrations used apparently inhibits completely the development of yeasts and molds. The data on proteolytic and lipolytic bacteria, while not conclusive, indicate that salt is not consistently more inhibitory to such organisms than is the acidity which normally develops in unsalted cream.

Microscopic examination of the cream revealed that the flora of salted cream is predominantly large paired and clumped cocci and a few sarcina types, instead of the small cocci in pairs and short chains, frequently followed by lactobacilli, which predominate in unsalted control lots of cream.

Salted cream apparently is non-corrosive to stainless steel of the types tested, but dairy metal and tinned copper are subject to noticeable corrosion and probably would not be suitable materials for equipment used in collecting and processing salted cream.

The addition of salt to cream as it is produced on the farm offers an apparently feasible method for considerable improvement of the quality of butter manufactured over a considerable section of the country. Acceptance by regulatory officials and by creamerymen must be obtained before the method can be placed in the hands of the cream producer.

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# NEW DEVELOPMENTS IN THE PHYSIOLOGY AND BIO-CHEMISTRY OF LACTATION; A REVIEW<sup>1</sup>

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The physiological and biochemical aspects of lactation may be divided broadly into three phases.

I. Development of the mammary glands.

II. Lactation.

III. Ejection of milk from the smaller gland recesses.

A comprehensive review of the literature pertaining to all of these would require far more space than is available. It is, therefore, proposed to deal only superficially with the endocrine aspects of gland development and the lactogenic hormones. The biochemical aspects will be dealt with more extensively but not exhaustively. It shall rather be the purpose to review the more recent pertinent literature with a view of establishing the present status of research in this field.

## I. DEVELOPMENT OF THE MAMMARY GLANDS

The literature pertaining to the growth of the mammary glands has recently been reviewed by Nelson (167), Turner (253), Folley (45), and Riddle (215, 216). For anatomical features of mammary development, see Turner (254, 255) and Espe (38). No attempt is made here to cover all of the areas reviewed in these papers, but an attempt will be made to incorporate the recent work with a view of establishing the status of the problem at the present time.

It is well established that mammary development in the normal animal is stimulated by ovarian hormones falling into two categories—estrogens and progesterins. The classical example is where the estrogen causes duct development and the progesterin causes alveolar development. While estrogen is needed for duct development in all species progesterin is not essential for the alveolar development in some species.

In the rat and the rabbit some alveolar development is observed, while for the guinea pig, Nelson (167), the goat, de Fremery (61) and Folley *et al.* (50), the monkey, Gardner and Von Wagenen (67), and the cow, Walker (262) and Turner (253), complete alveolar development is obtained from estrogen administration. Walker and Stanley, (262) obtained complete mammary development as well as initiation of lactation from diethylstilbestrol administration to an ovariectomized heifer, while Turner (253)

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obtained good udder development and considerable secretion from a spayed heifer with ovarian transplant.

The generally observed failure of mammary development in estrogen-treated hypophysectomized animals led to the conclusion that the hypophysis is involved. The main question is as to whether the estrogens and progesterins stimulate the hypophysis to form new hormones which are capable of causing mammary development or whether such formed hormones act synergistically with the sex hormones or whether normally present hormones of the hypophysis act with the sex hormones.

While Corner (31) suggested the pituitary might produce mammogenic hormones, evidence for mammogenic hormone formation by the pituitary through stimulation of the sex hormones comes chiefly from workers at Missouri. Gomez *et al.* (75,78) obtained mammary development in hypophysectomized guinea pigs by implantation of pituitaries from rats treated with estrogen and negative results from pituitaries of nontreated rats. Gomez and Turner (77) again reported that extracts from anterior pituitaries of pregnant cattle stimulated mammary growth in hypophysectomized rabbits and rats while the extracts from pituitaries of nonpregnant cattle were ineffective. Lewis and Turner (141) reported that the mammogenic factor in the pituitary is soluble in fat solvents and Lewis *et al.* (142, 144) reported on methods for biological assay also further confirming the effect of pregnancy on the pituitary content of the hormone. Reece and Leonard (206) found that implants of pituitaries from estrogen-treated and nontreated rats stimulated mammary development in hypophysectomized rats although they supported the mammogen hypothesis. In more recent work two mammogenic hormones are reported—mammogen I (Lewis (140)) for the development of ducts and Mammogen II (Mixner (162)) for the alveolar lobular development—and Mixner and Turner (163) have reported on a method for assaying the lobular alveolar growth promoting activity of the anterior pituitary.

In opposition to the "mammogen" hypothesis are the reports of failure to confirm the basic experiments leading to the development of the theory, the fact that estrogens have a local effect when applied topically, the possibility that the failure of mammary development in the hypophysectomized animal may be due to inanition and some evidence that the growth hormone is essential.

The results of Corner (31) and Nelson and Tobin (175), where crude pituitary extract together with estrogen caused mammary development in hypophysectomized rabbits and rats, respectively, might be explained by the supposition that the pituitaries they used contained the mammogenic hormone, and the negative results of Selye and Collip (222) could be explained by postulating the absence of the mammogenic hormone in the pituitaries they used. Nelson (168, 170) observed no difference in the development

of mammary glands in hypophysectomized rats from implants of pituitaries from estrogen-treated and nontreated rats. Similar results were obtained by Reece and Leonard (207). More recently Greep and Stavely (95) extracted bovine pituitaries according to the method of Lewis and Turner (141) and found the extract to be inactive while implants of the powdered pituitaries and extracted residue were active although the potency of the latter was somewhat reduced.

Topical application of estrogen to the nipple area of individual glands has been observed to cause development of the treated gland only with little or no effects upon the untreated one for: the rat by Lyon and Sako (151); woman by MacBryde (152); the goat by Folley *et al.* (51); the monkey by Speert (240); the guinea-pig by Nelson (171), and the bovine by Petersen *et al.* (186). These experiments may be interpreted as showing that the estrogen acts directly upon the mammary gland in a synergistic manner with other pituitary hormone or hormones.

Astwood *et al.* (4) suggested that the inanition state of the hypophysectomized animal may be a factor in the failure of mammary development, for intact rats restricted to a food intake comparable to that of the hypophysectomized ones resulted in failure of mammary gland response to estrogen. The report of Nathanson *et al.* (164) supports the view that under-nutrition is a factor in the failure of mammary growth stimulation to estrogen in hypophysectomy. Samuels *et al.* (220) force fed hypophysectomized rats by stomach tube so as to gain in weight but noted no effect on the mammary development. It should be noted, however, that while forced feeding caused increases in weight such might be due to adipose tissue increments and not to real growth.

That the growth hormone in conjunction with estrogen is needed for mammary growth is indicated by the work of Nathanson *et al.* (164), who noted that in eight of 24 hypophysectomized rats treated simultaneously with estrogen and a growth complex there were weight increases and marked mammary development. In six animals there were no weight increases and moderate gland development, and in ten animals with weight loss gland development varied from moderate to no effect. Greep and Stavely (95) observed body growth from pituitary powder implants although the amount of body growth and the extent of mammary development were not always correlated. More recently, Reece and Leonard (207) report on positive evidence of growth hormone effect with estrogen on mammary growth. Samuels *et al.* (220a), using a more highly purified growth hormone, corroborate the findings of Reece and Leonard, but contend other anterior pituitary hormones are also needed.

The observation of Gardner (65) that large doses of estrogens have an inhibitory effect on the mammary gland development is of great importance to workers in the field. Other important considerations are the effects of

other endocrines of which merely mention is made. Some of the androgens (see Folley (45) and Bottomley and Folley (15)) have mammogenic properties. The adrenal hormone, desoxycorticosterone, has been shown by Van Heuverswyn *et al.* (259) to be growth-promoting for the mammary gland of male mice. It must also be mentioned that Beall and Richstein (10) isolated progesterone from the adrenals, which may in part explain alveolar proliferation from estrogen administration alone. Another interesting observation was made by Butcher (25) in that the mammary glands of underfed adrenalectomized rats developed more rapidly than in the intact animals. Ovariectomy together with adrenalectomy produced similar results, indicating that the effect is not one in which the ovary is involved. The author has noted that the mammary gland of the thyroidectomized bovine develops much more slowly than in the normal. Because of the specific effects of the hypophysis upon other endocrine glands and in turn their probable effect upon the development of the mammary gland, complete development of the mammary gland in hypophysectomized animals no doubt will be found to require more than one fraction of the anterior pituitary.

## II. LACTATION

The complicated phenomenon of lactation may be divided, for ease of discussion into the following five parts: A. Endocrine; B. When milk is secreted; C. Equilibria between milk and blood; D. Relation of pressure to milk secretion; E. Synthesis of milk.

A. *Endocrine Factors.* The initiation and maintenance of lactation is dependent upon endocrines. From the literature it is apparent that the anterior pituitary, the thyroid and the adrenals are involved in complete lactation. Endocrines also play a part in inhibiting lactation. For reviews see Nelson (167), Turner (253), Folley (45, 46), and Riddle (215, 216).

1. *Anterior Pituitary.* Since Stricker and Greuter (245) in 1928 first discovered that injection of an aqueous extract of the anterior pituitary would initiate lactation in ovariectomized pseudo-pregnant rabbits a large number of experiments dealing with anterior pituitary lactogenic hormones have been reported. The literature is so large that only a small portion can be cited here. For further literature citations the reader is referred to the reviews (39, 43, 167, 216, 222, 254).

The main pituitary lactogenic hormone studied is known as prolactin (also as galactin by Turner and mammatropin by Lyons). Prolactin is universally accepted as a lactogenic hormone, but Riddle (216) rightly argues against calling it the lactogenic hormone, for, as will be shown, there are probably other lactogenic hormones. Although other methods of assay have been proposed (66, 166), the international unit is based upon the growth produced in the crop-gland of the pigeon. Therefore, prolactin refers specifically to crop-gland-stimulating activity.

The standard method of isolation of prolactin is that of Riddle and Bates (217, 218). While the chemistry is not known, in its purest form prolactin reacts like a protein (Riddle and Bates (217, 218), Bates *et al.* (8), Young (268), and McShan and French (156)).

While the anterior lobe of the pituitary is the chief source of prolactin, this hormone is also found elsewhere. Lyons and Page (150), Ehrhardt and Voller (37), and Turner and Meites (257) detected prolactin in the urine of lactating women; Lyons (148), in the urine of babies secreting witches milk and in the urine of normal human males. Leblond (136) has demonstrated prolactin in the blood of pregnant and lactating mares. Lessman (139), Ehrhardt (36), and Turner and Meites (257) have found the lactogenic hormone in placentas. A prolactin-like substance was found by Rabald and Voss (203) in normal beef and hog livers, but not in the liver of the horse.

That the anterior pituitary varies in its prolactin content as to species, physiologic stage, and age was first reported by Bates *et al.* (9). Chance *et al.* (29) report progressive increases in prolactin for the following species: horse (only 4% of the ox), swine, man, ox, and sheep. Reece and Turner (209) report a higher prolactin content in dairy than in beef cattle pituitaries. They also report a lesser concentration of the hormone in calves than in adults. In another report, these workers (210) found estrogen administration to increase the lactogen content of rat pituitaries. In guinea pigs, according to Reece (205), the hormone is greatest during lactation, next greatest in late pregnancy, followed by decreasing amounts in estrum, early pregnancy, and diestrus. Holst and Turner (110) found no increase of prolactin in early pregnancy, little in late pregnancy, but large increases following parturition of rabbits and guinea pigs. Ehrhardt and Voller (37) report two peaks of prolactin in the urine of post-partum women, one at menstruation and the other at mid-cycle corresponding to ovulation.

The question of the mechanism responsible for the secretion of prolactin to time with parturition has long been and still is a matter of speculation. In general, the theories advanced fall into five groups—1, the corpus luteum inhibits lactation; 2, the placenta inhibits lactation; 3, the lactation is inhibited by mechanical distension of the uterus; 4, estrogen secretion of pregnancy inhibits lactation, and 5, some unknown factor during pregnancy inhibits the production of prolactin.

The theory of the inhibitory action of the corpus luteum is supported by the work of Drummond-Robinson (34) and Asdell (2), who reported that removal of the corpora lutea from pregnant goats with well-developed mammary glands resulted in lactation. Selye *et al.* (224) observed that ablation of the corpora lutea in rats with well-developed mammary glands and treated with the luteinizing hormone also resulted in lactation. Hammond (101) as early as 1917 advanced the theory that the corpus luteum inhibited lactation.



The reports of Anselmino and Hoffmann (1) and Folley and Kon (48), who injected progesterin into lactating animals and failed to inhibit lactation, must be cited as evidence against the theory, although Anselmino and Hoffman suggested that some other corpus-luteum factor than progesterin is responsible for the inhibition. Lactation in the human, cow, goat, and other species during pregnancy would also indicate that the corpus luteum is not the chief inhibitory factor.

In support of the placental inhibition theory are the reports of Frankl (59), Nelson (165), and Smith and Smith (238). These workers observed inhibition of lactation when placentas were retained, and Frankl also obtained inhibition of lactation by placental implantation. Litt (146), however, was unable to observe any inhibitory effect on lactation by the implantation of placentas in rabbits as was also Selye *et al.* (224) with mice. Other objections to the theory come from the lactations during pregnancy and the often observed fact the retained placentas in cattle, at least, do not completely inhibit lactation in that species.

That the mechanical distension of the uterus may inhibit lactation finds support in the report of Selye *et al.* (224), as when the young in rats were removed by Caesarian section and the uteri filled with paraffin, lactation failed, although Bradbury (17), using the same technique, could not confirm the work of Selye and others. Freud and Wijsenbeck (62) found that transferring rat fetuses from the uterus to the abdomen inhibited lactation until removed therefrom.

That estrogens inhibit lactation is supported by too many reports to be reviewed here. Among the many papers suppression of lactation by estrogen administration has been reported for the mouse (122, 219), the rat (48, 209), the cow (42, 46), the guinea pig (84, 123, 135), and woman (58, 128, 137, 204, 267). It must be noted that heavy doses of estrogen are required for inhibitory effects on lactation; in fact, larger amounts than would be present in pregnancy. Nelson (167) advances the theory that estrogen during pregnancy acted through two channels: (a) by suppressing the secretion of the lactogenic hormone by the hypophysis, and (b) by direct action on the mammary gland. Turner and Meites (257) point out that this cannot account for lactation in pregnancy in the several species where this phenomenon is observed nor does it explain the initiation of lactation when diethylstilbestrol is used for mammary development such as has been observed by Folley *et al.* (50) and Lewis and Turner (143) for the goat and by Walker (262) for the cow. The observations of Folley and Watson (49), Folley *et al.* (52), and Spielman *et al.* (241), that injection of large doses of diethylstilbestrol into lactating cows did not inhibit milk production but merely altered the composition of the milk, suggest some species differences in response to estrogen as diethylstilbestrol has been shown to inhibit lactation in other species. It must also be stated that this theory of Nelson does not agree with the obser-

vations of Reece and Turner (209), who reported increases in the prolactin content of the pituitaries following estrogen administration.

Turner and Meites (257), after criticizing the theories advanced relative to the initiation of lactation and presenting evidence to show that pregnancy does not lower the lactogenic hormone of the pituitary, propose that copious lactation can be initiated only when (a) there is a well-developed mammary gland present and (b) when there is a high lactogen content of the anterior pituitary.

Since it is well established that lactation can take place during pregnancy, and accepting the suggestion of Turner and Meites (257) that high lactogen content of the pituitary is essential for copious lactation, it appears to the reviewer that initiation of lactation at the time of parturition is not due to the removal of some inhibitory factor but rather is due to the introduction of some stimulating factor. This stimulating factor may well come from the posterior pituitary as the oxytocic principle of that gland is secreted at the onset of labor. The observations by Reece and Turner (208) of the effect of suckling upon the prolactin content of the pituitary support such a hypothesis as does also the theory of Ely and Petersen that milk is ejected from the alveoli through the action of oxytocin in turn liberated by the milking stimulus. The recent report by Hooker and Williams (111) that injection of the lactogenic hormone retarded involution of the mammary gland emphasizes the dual role of this hormone—maintaining the mammary gland as well as stimulating lactation.

That prolactin is not the only anterior pituitary hormone needed for the initiation and maintenance of lactation is indicated by a number of experiments (Folley and Young (53, 54, 56)). The earlier work with success of initiating and maintaining lactation in the hypophysectomized animal can be explained by assuming that the extracts used were relatively crude. This seems to be the explanation for the success of Riddle *et al.* (218) with rats; Stricker and Grueter (245) with rabbits; Lyons *et al.* (149) and Housay (112, 113) with the dog; and McPhail (155) with the cat. Failures of Nelson (167) and Selye *et al.* (223) with the use of crude anterior pituitary extracts are difficult to explain. Later, however, Nelson and Gaunt (172) found that a purified preparation failed, while a crude extract of the anterior pituitary succeeded, in initiating lactation in hypophysectomized guinea pigs. Gomez and Turner (71), also working with hypophysectomized guinea pigs, found their purified preparation of prolactin failed to initiate lactation.

Thyroxin has been ruled out as the missing hormone by Gomez and Turner (75, 76), as the simultaneous administration of thyroxin and purified prolactin failed to initiate lactation. Leonard and Reece (138), however, report that thyroidectomy of rats resulted in a thickening of the ducts and an increase in the number of end and lateral buds to indicate an effect

of the thyroid upon the growth of the gland. Atrophy of the adrenals accompanies hypophysectomy, and it is not surprising that simultaneous administration of the adrenocortical hormone and prolactin is effective in initiating lactation as has been reported by Gomez and Turner (71, 73, 74) and by Nelson and Gaunt (172, 173, 174). Climenko and McChesney (30) observed that injection of epinephrin with prolactin and adrenal cortex hormone augmented milk flow.

In addition to laboratory animals, injections of anterior pituitary extracts have been reported for the cow, goat, sheep and the human. Grueter and Stricker (98), Evans (39, 40), Asimov and Krouze (3), and Folley and Young (53, 54, 55) injected rather crude extracts into lactating cows and observed significant rises in milk production, Evans (40) reporting as much as 25 to 50 per cent increases. In the main, the effects were temporary and most pronounced in cows during the downward trend of the lactation curve, and some cows did not respond at all. Asimov and Krouze reported the injections to be more effective in the first half of the lactation. Stockklauser and Daum (244) found that injections of anterior pituitary extracts caused a decline in milk production of cows. Experiments by Folley and Young (54, 55) showed that while "purified" preparations of prolactin caused increases in milk production, preparations containing the glycotropic factor were more effective even though such extracts contained less of the pigeon-crop-gland stimulating factor.

For goats, anterior pituitary extracts have been shown to stimulate milk production similarly to the cow by Grueter (97), Evans (39), and Asdell *et al.* (2). For sheep, Kabak and Kisilstein (125) have reported stimulation of lactation following administration of anterior pituitary extracts.

Prolactin has been used with conflicting results in attempts to increase lactation in women. Kenny and King (131) report 74 per cent of 43 women responded with satisfactory lactation following injections of prolactin. Kurzrok *et al.* (133) also report favorable results. On the other hand Stewart and Pratt (243) reported negative results with 14 women and Werner (265) describes some severe reactions from prolactin injections.

2. Thyroid. Since the thyroid regulates body metabolism, this gland would be expected to exert a marked influence over lactation. Jones (121) points out that the thyroid hormone may influence milk secretion in three ways: 1, by affecting the level of the blood precursors of milk; 2, by influencing the rate of blood flow through the gland, and 3, by a direct effect on the gland secretory cells. Studies on the thyroid-milk-secretion relationships have been made by two general methods—thyroidectomy and administration of the thyroid hormone to intact animals.

The reports on the effects of thyroidectomy are conflicting for reasons that are obscure. For lactating cows, Graham (84) reported only a slight decrease in milk production following thyroidectomy, while Spielman and

Petersen (242) have observed not only a slowing of the development of the mammary gland but a complete cessation of lactation in 180 days following thyroidectomy. Trautmann (252) reported thyroidectomy of goats resulted in a significant decrease in milk yield, while Hibbs *et al.* (105) obtained lactation for more than a year in the thyroidectomized goat. Nelson and Tobin (176) and Nelson (169) have reported observing no effect of thyroidectomy in the rat on lactation while Folley (44) reports marked diminution of milk secretion following the operation on this species. Dragstedt *et al.* (33) report apparent normal milk secretion in the thyroidectomized bitch provided that tetany was prevented.

In the administration of thyroid and thyroxine to intact animals there is better agreement as to its effect in increasing milk secretion. Graham (84, 85), Jack and Bechdel (118), Folley and White (57), Herman *et al.* (103), and Hurst *et al.* (115) have reported on increased milk production in the cow following either thyroid feeding or injection of thyroxine. De Fremery (60) reported opposite results in goats with thyroxine, which Folley (45) explains as probably being due to too large doses. All observing increased milk production in the cow, with the exception of Jack and Bechdel (118), also observed increased fat percentage, pointing to the thyroid being especially involved in milk-fat synthesis. Folley and White (57) observed that thyroxine injections raised milk production to a peak, and with continued injection milk production declined at a normal rate but remained at the higher level. Attention is again called to the reports of Folley and Young (55, 56), where anterior pituitary extracts containing the thyrotropic hormone are more effective in increasing lactation than preparations without this principle. However, Grumbrecht and Von Dusterlo (99) reported the thyrotropic hormone decreased lactation in the guinea pig. Di-iodotyrosin has been reported as increasing milk production in the guinea pig by Grumbrecht and Von Dusterlo (99) and in women by Küstner (134). Turner (256) has reported oral administration of iodized skim milk to increase milk production in goats comparable to thyroid, the active principle being termed thyrolactin (256). Heathman and Turner (104) have reported an assay method for thyrolactin.

Graf *et al.* (83) noted no effect upon amount of milk by administration of small doses of dinitrophenol but marked changes in composition. On large toxic doses there was a diminution in milk with more marked changes in composition. Brower and Martin (19) observed marked declines in milk flow as well as changed composition when this drug was administered to goats.

3. Adrenals. There is an increasing literature that the adrenal cortex hormone is needed for normal lactation, as ablation of the adrenals has been reported by Carr (27), Swingle and Piffner (249), Gaunt (69), Britton and Kline (18), Nelson and Gaunt (173), Gaunt and Tobin (70).

Brownell *et al.* (23), and others to prevent normal lactation. The need for the adrenal cortex hormone in conjunction with prolactin to initiate lactation in hypophysectomized animals has previously been cited. The mode of action of the adrenal cortical hormone is speculative. Brownell *et al.* (23) suggested a special lactation hormone in the adrenals but Folley (42) and Nelson and Gaunt (173) suggest that the action of the adrenals is indirect. They propose that the general upset in metabolism in the adrenalized animal is responsible for the adverse effects on lactation. The work of Climenko and McChesney (30) showing that epinephrin administration augmented the effect of the cortical hormone and prolactin in hypophysectomized animals is of interest and adds emphasis to the fact that complete lactation is dependent upon the interaction of a number of endocrine secretions.

B. *When Milk Is Secreted.* The older idea that milk secretion takes place in two phases, one in the interim between milking and the other at milking time due to the milking stimulus, was supported, among others, by the reports of Isaachsen (117), who stated that cows producing 5 to 6 kilos per day secreted 2 to 2½ kilos during milking, and of Maxwell and Rothera (157), who concluded at least 40 per cent of the milk produced by rats was secreted during the nursing act. The capacity of udders for containing all the milk produced at a milking has been reported by Gaines (63) for goats and by Zwart (270) and Swett (247) for cows by injecting solutions into the udder after withdrawal of the milk. Zwart (270), Gaines and Sanmann (64), Gowen and Tobin (79), Petersen *et al.* (190), and Swett *et al.* (248), slaughtered cows before milking at the regular milking time and estimated the amount of milk in the udder by either post-mortem milking or by chemical analysis of the udders, and were able to account for all or nearly all the milk predicted on the basis of pre-slaughter records. On the basis of the evidence at hand, it must be concluded that the milk is secreted in the interval between milkings and that there is no evidence for increased rate of secretion during the milking act. The reports of Shaw and Petersen (233) would indeed indicate that during the milking act there is complete cessation of secretion, for the mammary venous blood at this time contains more fat, calcium, and phosphorus than the arterial blood.

C. *Equilibria between Milk and Blood.* While milk is isotonic with blood, each having an osmotic pressure of 6.6 atmospheres, the two substances are not in equilibrium. According to Simms (225), milk contains, on a molar basis, 20 times the fat, 40 times the sugar, 7 times the potassium, 14 times the calcium, 4 times the magnesium, and 7 times the  $\text{PO}_4$  content of blood. On the other hand, blood contains 2 times the protein, 8 times the sodium, and 4 times the chlorine content of milk. Simms dialyzed milk against blood serum and found a shift toward the same concentration for the salts, but magnesium and calcium were still 2.1 and 4.4 times as concentrated, respectively, in the milk as in the serum at the end of the dialysis.

For the above-mentioned constituents the mammary gland acts in a selective manner, preferentially absorbing some and repelling others. For other substances the mammary gland behaves as a permeable membrane in which the levels are the same in both the blood and milk. Peskett (183) has shown that urea concentration of blood and milk are identical. The same is probably true for other normal blood constituents such as uric acid, creatine and creatinine. Many substances administered orally or inhaled will likewise pass from the blood into the milk.

Any disturbance within the udder, such as mastitis, introduction of foreign substances, and continued pressure within the gland, will interfere with the normal behavior of the gland and cause it to behave more like a simple membrane. Petersen and Rigor (194) studied the effect of leaving milk in the udder for 24, 36 and 120 hours and found the total solids, protein, pH, and ash values to increase while the lactose decreased. Garrison and Turner (68) noted that suspending milking for 24 hours tended in the same direction and also observed the catalase and chloride content of the milk to increase. Porcher and Muffet (202) observed that the casein content of retained milk declined and the globulin increased.

Udder irrigations have shown the mammary gland to behave contrary to that expected on a basis of known physical laws. Filling the udder with distilled water immediately after milking decreased production but slightly according to Petersen and Rigor (195) and Garrison and Turner (68) and lowered the lactose and increased the total solids, protein, pH, and ash contents slightly. Hueker and Lee (114) found a 0.12 per cent sodium chloride solution to have little effect. Increasing the concentration of salts, sugar, and a mixture of salts intensified the disturbance, which lasted for several days after the injections. When hypertonic solutions were injected (195) it was observed that practically all of the injections were resorbed in 12 hours where it was expected that water would pass from the blood into the more concentrated solution to increase the volume. Still harder to explain is the effect of reinjecting the milk withdrawn from the udder, which has been reported by Jackson and Rothera (120), Davidson (32), and Garrison and Turner (68) to have the same effects as injecting salt solutions of about isotonic concentration. Petersen and Turner (201) have found that injection of blood serum and a 6 per cent gum acacia concentration in Ringer's solution had the severe effects of a hypertonic salt solution.

Oral or subcutaneous administration of toxic doses of dinitrophenol (19, 83) have been shown to increase the permeability of the mammary gland to the sodium bicarbonate of the blood, which phenomenon is responsible for an increase in the pH of the milk.

It should be noted that with all injections, or leaving the milk in the udder, there is a great increase in the cell count of the milk; or, in other words, the characteristics of milk retained in the udder or following injection of any kind into the udder are similar to those of mastitic milk.

As a result of the studies on maximum pressures developed and equilibria phenomena established in the udder and ultimate resorption of the milk by refraining from milking, Wayne *et al.* (263) reported that no harmful effects could be observed from drying off cows by suddenly stopping milking.

*D. Relation of Pressure to Rate of Milk Secretion.* Not only is all milk secreted in the interim between milkings but due to the intra-alveolar pressure developed by the accumulating milk the rate of secretion diminishes with time and in high producing cows may be completely stopped before milking. Neusch (177), Isaachsen (117), and Tgetgel (251) reported measurements of the milk pressure within the gland cistern at milking and Petersen and Rigor (193) measured the maximum pressure developed by not milking cows. While in general these measurements showed that pressure increased with the accumulation of milk, and that with not milking a maximum pressure is developed, followed by a decline, the method does not measure the intra-alveolar pressure and is also affected by the "letting down" of milk phenomenon. Petersen and Rigor (193) maintained constant air pressures in the gland during the interim between milkings and found a progressive decrease in the rate of milk secretion as the air pressure was raised from 10 mm. to 25 mm. of mercury, when secretion was stopped. Garrison and Turner (68) used oxygen pressures at 10 and 40 mm. mercury levels and reported small secretion at the higher level, which may be due to residual milk in the gland at the time the pressure was applied, for the milk had a high fat content.

The inhibitory effect of pressure upon the rate of secretion is of great practical importance and is the chief explanation for the increase in milk production obtained by more frequent milking. The report of Ludwick *et al.* (147), where milking one side of the udder 3 times daily increased milk production as much as 16 per cent without affecting the production on the other side milked but twice daily, would indicate that the effect of more frequent milking is due to the lowering of the pressure within the gland and not to the more frequent stimulation of milking.

*E. The Synthesis of Milk.* The synthesis of the various ingredients of milk has been studied by determining the uptake of probable blood precursors by the mammary gland, by altering the level of the probable precursor in the blood, by analysis of the mammary glands, and by perfusing the surviving mammary gland.

Kaufmann and Magne (126) first used the technique of determining the difference between jugular blood and mammary vein blood which has since been used by a number of investigators. The results are erroneous, as pointed out by Blackwood and Stirling (13), for first it is assumed that the maintenance requirement of the head, drained by the jugular, is the same as that of the udder. Secondly, the composition of the jugular blood is

greatly influenced by the secretion of saliva. Blackwood and Stirling introduced the technique of using simultaneously taken arterial blood and mammary venous blood for determination of blood precursors of milk. They used radial artery blood. Since then arterial blood has been taken from the left ventricle of goats by Lintzel (145), the internal iliac artery, through the rectal wall, by Graham *et al.* (90), the internal pudic artery, through the vaginal wall, by Maynard *et al.* (159), and by exteriorizing the carotid artery in goats by Graham *et al.* (92). The arterio-venous difference technique, while superior to the Kaufmann-Magne method, is still subject to a number of criticisms (188). The samples must be taken simultaneously and without disturbance to the animal, for Shaw and Petersen (232) have shown as much as 14 per cent concentration of blood passing through the mammary gland during excitement. The returning lymph from the mammary gland undoubtedly alters the composition of the blood and is unaccounted for by this method, and there is no means of knowing what are the maintenance requirements of the gland itself. To obviate the effect of excitement, Reineke *et al.* (212) have suggested anesthetizing the animal. However, such procedure is found to have other effects upon the general body metabolism. Perfusion experiments described by Petersen *et al.* (200), while also subject to criticism, obviate some of the criticisms of the in-vivo experiments. Among the advantages given by Petersen *et al.* (199) for perfusion in studying milk secretion are: elimination of the metabolic factors of the body, effect of depletion of some precursor upon milk secretion, and the addition of certain substances to the perfusion that cannot be done in the intact animal. A combination of in-vivo and perfusion studies is, therefore, desirable. Study of the analysis of the mammary gland, which has not been resorted to, to any great extent, offers possibilities of contributing much to the knowledge of the metabolism within the gland.

Before proceeding to a discussion of the literature pertaining to the synthesis of the various milk ingredients there are a few problems of general importance in milk secretion to consider. First is the ratio of blood flow through the gland to the amount of milk. Assuming glucose to be quantitatively converted into lactose and on the basis of the glucose uptake by the mammary gland of cows, Graham *et al.*, (89) calculated 500 volumes of blood passes through the gland for each volume of milk produced. On the same basis Lintzel (145) calculated 256 volumes of blood per volume of milk for goats. Shaw and Petersen (231) found the ratio of blood to milk (in cows) to be 387 to 1 on the basis of calcium uptake; and 391 to 1 on the basis of combined glucose and lactic acid uptake assuming the latter two are quantitatively used for lactose synthesis. Direct measurements of blood flow by Graham (86) by means of a thermostromuhr gave only about one half the values. Jung (124) found using stromuhr essentially the same. The disagreement among the reports of ratio of blood flow to milk is difficult



to explain. It is believed that determination of the use of calcium by the mammary gland is the most accurate means of measurement and that, therefore, in the cow, the ratio is about 400 volumes blood per volume of milk.

Only one paper has appeared upon the difficult task of calculating the energy used by the mammary gland—that of Graham *et al.* (87), who estimated about 10 per cent of the total energy uptake of the gland from the blood was used by the gland itself.

1. Fat Metabolism in the Mammary Gland. Theoretically it is possible for the milk fat to come from blood fat, protein, or carbohydrate. Of the blood fats, neutral fat, phospholipids, or sterol esters must be considered as the probable precursors of milk fat. The evidence now points to neutral fat as the chief, if not exclusive, blood precursor of milk fat. Foa (41) and Petersen *et al.* (192) perfused mammary glands with oil emulsions, the former finding the oil in the milk while the latter observed none of the stained fat in the milk but reported 3 per cent of the dye was found in the gland fat. In each case, therefore, the gland took up neutral fat. Since then neutral fat of the blood has been shown to be taken up by the mammary gland by Blackwood (12), Lintzel (145), Graham *et al.* (89), Maynard *et al.* (159), Shaw and Petersen (233), and Voris *et al.* (261), all comparing arterial blood with mammary vein blood. These workers, together with Aten and Hevesey (5), have shown that the mammary gland does not take up phospholipids as was advanced by Meigs *et al.* (160), using the Kaufmann-Magne technique. Aylward *et al.* (6) reported iodized tryglycerides fed to produce lipemia in cows caused but little iodine to be detected in the phospholipid fraction of the blood and large increases in the iodized fat of the milk.

Shaw and Petersen (233) have shown that the mammary gland takes up more than enough neutral fat from the blood to account for the milk fat and support the suggestion of Hilditch and Thompson (107) and Hilditch and Paul (106) that the short chain fatty acids come from a breakdown of oleic tryglycerides. Shaw and Knodt (227) have shown that the mammary gland uses  $\beta$ -hydroxybutyric acid, but for what purpose is still speculative, although these authors are inclined to believe it is used for energy purposes. Shaw and Petersen (233) have reported that the uptake of fats from the blood is practically nil during milking and increases for several hours after milking.

In further support of the breakdown of higher fatty acids for the formation of the lower fatty acids of milk are the reports of Petersen *et al.* (191) and Gowen and Tobey (80) that the fat in an active mammary gland is intermediate between body fat and milk fat. This would indicate that the fat stored in the gland is gradually changed to milk fat. The location of free fatty acids in the basal part of the secretory epithelium by Kelly and Petersen (130) and the detection of lipase in the active mammary gland also tend to support the breakdown of higher acid theory.

The finding of a respiratory quotient of the mammary gland above unity by Graham *et al.* (87) and Reineke *et al.* (212) led them to postulate the formation of fat from carbohydrate, although their data of uptake of carbohydrate from the blood were not equal to the requirements for the production of lactose. Shaw (226) reported on somewhat lower respiratory quotients on perfused udders. It is generally agreed that the respiratory quotient by itself is not reliable in deducing the type of metabolism that takes place in an organ. (See Soskin (239).)

The synthesis of milk fat seems to be more or less independent of the other ingredients of the milk, as the fat content may be increased or decreased without affecting the amount of milk. The literature on the effects of the diet upon milk fat is entirely too large to review here, but it may be said that the character of the fat is greatly altered by the type of fat fed (158) and by inanition (237). Fat content of milk may be increased by increasing certain fats in the diet and decreased by cod liver oil feeding (184). Administration of thyroid, dinitrophenol, and diethylstilbestrol causes increases in fat content of the milk without necessarily affecting the quantity of the milk. Daily secretion of milk fat is more persistent than that of milk, with the decline of milk with the advance of lactation, there is an increase in fat percentage.

2. Lactose Synthesis. That blood glucose is taken up by the mammary gland has been shown by arterio-venous differences observed by Blackwood and Stirling (14), Lintzel (145), Graham *et al.* (87, 89, 90), Shaw *et al.* (227), and others, all of whom postulated blood sugar as being the precursor of milk sugar. Hypoglycemia has been produced by insulin administration by Petersen *et al.* (189), Brown *et al.* (20), Gowen and Tobey (82), Guisti and Rietti (100), Nitescu and Nicolescu (179), Macchiarno (154), and Bucciardi (24), all noting decrease in lactose content of the milk following insulin use. Similar results have been reported following phloridzin administration by Paton and Cathcart (182) and Gowen and Tobey (82). Lowered lactose contents of milk following inanition with hypoglycemia has been reported by Overman and Wright (181) and Gowen and Tobey (81).

Hyperglycemia has been produced by intravenous injections of glucose by Brown *et al.* (21), Nitescu (178), and others, by introduction of large quantities of glucose into the stomach by Whitnah *et al.* (266), by resorption of sugars introduced into the mammary gland by Brown *et al.* (22), by thyroxine injection by Jones (121), and by implantation of adrenalin tablets by Bottomley *et al.* (16). Petersen and Boyd (187) infused glucose through the intact mammary gland through the external pudic artery to greatly increase the glucose content of the blood going through the mammary gland. In these experiments increased lactose content of the milk was reported with observed hyperglycemia except by Brown *et al.* (21) and Petersen and Boyd. Brown *et al.* consider the intravenous injection of

sugar as of doubtful value in studying lactose synthesis because of other effects of the injections. In the case of failure, of lactose increase due to infusion of large quantities of glucose in the external pudic artery, Petersen (185) has suggested that increased amounts of lactose may be formed in the gland as the result of more available glucose, but, because of increased osmotic pressure, the excess (above normal) lactose diffuses back into the blood. This explains why it is possible to reduce lactose markedly by hypoglycemia, while it can be increased but slightly by hyperglycemia.

That glucose is the only precursor of lactose is to be doubted as there is good evidence that lactic acid is involved in lactose synthesis. Graham (86) and Shaw *et al.* (227) reported that the mammary gland takes up lactic acid from the blood and the latter calculated the glucose plus lactic acid uptake by the mammary gland, would be about enough to account for the lactose while the glucose uptake alone is not adequate. The possibility of glycoproteins being a source of sugar to the mammary gland complicates matters still further, as Reineke *et al.* (213) have reported lactating glands to take up glucose equivalent to more than 2 mgm. per 100 ml. of plasma from this source.

Several have attempted to establish the lactose precursors by the method of synthesis. Of these, only Grant (93, 94) and Petersen and Shaw (196) have definitely identified lactose as the end product. Grant demonstrated lactose formation from glucose added to fresh slices and observed no increase from the addition of hexose monophosphates or phosphoglycerate. Petersen and Shaw, however, could not demonstrate lactose formation from glucose and macerated mammary gland tissue, but did so when lactic acid was added. Since Petersen and Shaw (197) demonstrated that the active mammary gland contains 0.20 per cent glycogen, the question is raised as to the possibility that the breakdown of glycogen is essential for the formation of lactose and that Grant's mammary slices contained considerable glycogen. Weinbach's (264) postulation that the mammary gland contains a non-reducing precursor for lactose fits in with such a conjecture.

3. Nitrogen Metabolism in the Udder. Less is known about the synthesis of milk proteins than any of the other ingredients of milk. The only safe statement at the present time is that the precursors of milk proteins must be some of the blood nitrogen compounds, with some good evidence to indicate that blood globulin is involved. Cary (28), using the Kaufmann Magne technique, was the first to claim that blood amino acids were the precursors of milk protein, which was supported by Blackwood (11), using radial artery and mammary venous bloods for analysis. Graham *et al.* (87, 91), Shaw and Petersen (229, 231), and Reineke *et al.* (211), however, showed that the uptake of amino acids by the mammary gland was inadequate to account for the milk proteins, and, further, Graham *et al.* (88) and Shaw and Petersen (229) reported that the mammary gland produced

urea in quantities to account for the amino acid nitrogen taken up by the gland. Shaw and Petersen (229) reported that the mammary gland did not take up uric acid, creatine, and creatinine. Graham *et al.* (91) and Reineke *et al.* (211) claim that the mammary gland takes up considerable quantities of globulin from the blood, and, recently, Reineke *et al.* (213) have reported that glycoproteins, which are globulins, are taken up in appreciable quantities. The latter, together with the report of Jackson and Gortner (119) that in lactating glands globulin predominates and in non-lactating glands albumin predominates, indicates that probably globulin of the blood is a precursor of the milk proteins. The fact that in the blood there may be reversible shifts from albumin to globulin must be recognized as a possible explanation for an apparent uptake of globulin from the blood.

The report of finding arginase in the mammary gland by Shaw and Petersen (230) does not simplify the picture. Does the arginase merely split off urea from incoming arginine? Or, as a result of deamination of amino acids, is urea formed from  $\text{CO}_2$  and ammonia? Much more work is required before these questions and other fundamental questions relating to milk protein formation can be definitely answered.

While arterio-venous blood analysis has contributed much to a better understanding of milk synthesis, merely ascertaining the uptake of a blood constituent by the mammary gland does not necessarily prove such is a definite precursor for a definite milk constituent. Before any blood constituent can be definitely established as a precursor for any milk constituent, its metabolism in the udder must be determined. To date, very little work has been done in this direction. The synthesis of lactose by the mammary gland from glucose and lactic acid is a small beginning; even that does not go far enough, for it is possible and even probable that both must go through glycogen.

The reports by Kelly (129) and Virtanen (260) of finding lipase in the mammary glands suggest that studies be made on the mammary gland, involving the use of this enzyme. Phosphatase, reported to be present in mammary glands by Kay (127) and found to be similar to the kidney phosphatase and to be present in large quantities in the mammary gland by Folley and Kay (47), suggests the great importance of phosphorus metabolism in milk synthesis. Folley and White (57) suggest the mammary gland synthesizes large quantities of phosphatase, part of which is excreted in the milk.

Very little work has been reported on the proteolytic enzymes in the mammary gland. Tateyama (250) reported finding a peptidase in human breasts, and Shaw and Petersen (230) have reported finding arginase in bovine glands.

Of carbohydrate enzymes, Grimmer (96) and Tateyama (250) have reported finding amylase, and Kleiner and Tauber (132) have reported finding maltase.

### III. THE EJECTION OR "LET DOWN" OF MILK

Many postulations have been advanced to account for the commonly observed response to the milking or nursing act, in which the gland becomes turgid. The oldest postulate is that most of the milk was secreted during the milking act as the result of a nervous stimulus. The reports of Swett *et al.* (248), Petersen *et al.* (190), Gaines and Sanmann (64), and others, where all or nearly all the milk expected was obtained from excised udders, rather definitely established the fact that all the milk drawn at a milking is present at the beginning of a milking.

Hammond (102) advanced the hypothesis that the milk was forced out of the alveoli by erection. The milking stimulus reflexly caused the teat and gland to become engorged with blood, according to this hypothesis. The regression in the size of the udder with the progress of milking must be taken as evidence against the validity of the theory.

In another theory, supported by the arguments of Zeitzschmann (269), a contractile mechanism in the udder and teats is postulated as being responsible for the retaining of the milk. The failure for "let down" of the milk is, therefore, a positive act according to this postulate.

That the nervous system is involved in milking is supported by a number of experiments, but how the nerves are involved has only recently been clarified. Sympathectomy, by Ingelbrecht (116), Selye (221), Seyle *et al.* (224), Bacq (7), Cannon and Bright (26), and Simeone and Ross (234), was observed in most cases to inhibit lactation to a greater or less extent, but, in some cases, not until after the next gestation following the operation. In the case of Ingelbrecht, resection of the spinal cord was made so the front breasts of rats were innervated and the posterior denervated. He observed that the young nursing the posterior breasts died of starvation unless the front breasts were nursed at the same time. Ribbert (214) observed that transplanted mammary tissue would function; such transplant being free of nervous connections. Ely and Petersen (35) resected the inguinal nerve trunk to the one side of the udder of cows and noted no effect upon either the growth of the udder or the amount of milk let down. Petersen and Shaw (198) observed that cows under anesthesia failed to let down their milk even though the udders were filled with milk, as proven by the let down of milk to intravenous injections of the oxytocic hormone. With the exception of the last, all of these experiments indicate the involvement of the nervous system, which can best be explained after considering the hormonal relationship to milk "ejection."

The posterior pituitary extract has been studied in its effect upon milk secretion and reported to be either a galactagogue or to cause more complete evacuation of the milk in the gland, by Ott and Scott (180), Gaines (63), Simpson and Hill (235, 236), Hill and Simpson (108, 109), Hammond (101), Turner and Slaughter (258), McCandlish (153), and others, in

various species. For these experiments the extract contained both the pitressor and oxytocic principles. Since Kamm succeeded in separating the two fractions, Ely and Petersen (35), on the basis of experimental work, concluded that the oxytocic fraction causes a contraction of the musculature shown by Swanson and Turner (246) to be found around the alveoli. Ely and Petersen suggest that the sensory endings on the teat and afferent pathways are the only nerves needed for milking response. A stimulation of the nerve endings on the teat and udder, carried upward through the central nervous system, caused the oxytocic principle to be ejected from the posterior pituitary into the blood, whence it is carried to the udder. This reflex may become conditioned to many other stimuli, such as feeding, rattling of milking utensils, washing of the udder, etc. Gomez (72) has reported upon the need for posterior pituitary extract for lactation in the hypophysectomized rat in support of the theory.

Ely and Petersen (35) noted that fright inhibited a response to the milking stimulus. Miller and Petersen (161) have reported that stimulating cows to let down their milk by washing the udder 20 minutes before milking resulted in incomplete milking and gradual "drying off." Taking too long to milk (more than 8 minutes) had the same effect. Almost any factor which attracts the attention of the cow during milking will detract from a complete response to the milking stimulus.

From the foregoing, it is apparent that research work during the past decade has contributed much to a better understanding of the fundamentals underlying the phenomenon of lactation. Crucial evidence for all the pituitary factors involved in mammary gland development is still wanting. Much more work needs to be done on the blood precursors and on the mode of synthesis of the various milk constituents in the mammary gland before the complete picture of milk secretion is at hand. The blood precursors of milk protein and particularly their synthesis in the mammary gland is obscure as is also the forces in the gland secretory tissue that select certain constituents from the blood and hold back others. The phenomenon of ejection or "let down" of milk, although not completely solved, is now fairly well established, and this knowledge promises to contribute much to better milking practices.

Lastly comes the important problem of practical application of the newly found knowledge, an attack of which has scarcely begun. Will it be possible to increase mammary growth and subsequent lactation through application of the knowledge now at hand? Will it be possible to increase and prolong lactation at a higher level due to the administration of lactogenic substances? These and other questions can only be answered after much more research has been done.

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# American Dairy Science Association Announcements

## MICHIGAN INVITES YOU

*To the Officers and Members of the  
American Dairy Science Association:*

Michigan State College is looking forward to the meeting of the American Dairy Science Association here on its campus next June.

This, the oldest of the agricultural colleges, has grown into a great university but continues to regard as its most important function service to the agricultural interests of the state and nation. There have been four heads of the dairy department at Michigan State College since the creation of that department, and each of them has at one time served as President of your Association: A. C. Anderson in 1918, O. E. Reed in 1924, Dean E. L. Anthony in 1931, and Earl Weaver in 1938.

This college has unusual facilities for entertaining your group and is setting aside the use of the auditorium and certain dormitories and such other facilities as are required. It will be a good time to bring the members of your families to enjoy the early summer beauty of Michigan.

We are looking forward to your coming and extend to you a most cordial welcome. All the facilities of Michigan State College will be made available for your use and enjoyment.

(Signed) JOHN A. HANNAH  
*President, Michigan State College*

THIRTY-SEVENTH ANNUAL MEETING, MICHIGAN STATE  
COLLEGE, EAST LANSING, MICHIGAN, JUNE 22-25, 1942

### FIRST CALL FOR TITLES

Titles of papers to be presented should be in the hands of the program committee not later than April 1, 1942. Program chairmen are as follows:

Extension section—J. F. Kendrick, Bureau of Dairy Industry, U.S.D.A.

Manufacturing section—E. H. Parfitt, Evaporated Milk Ass'n., 307 N. Michigan Blvd., Chicago, Illinois.

Production section—H. A. Herman, University of Missouri.

General chairman—H. W. Cave, Oklahoma A. and M. College.

Titles should be sent to the section chairman concerned.

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## A PROPOSED SCORE GRADE METHOD OF DETERMINING THE QUALITY OF MILK\*

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The quality of milk is of great interest to the consuming public and the dairy and other food industries. Continued efforts have been made to devise score cards by which the quality of milk can be evaluated. Such cards have been numerous and varied in type and have often required laboratory determinations to complete the scoring. Score cards of this type have only partially fulfilled the need of the consumer, the teacher, the grader on the factory platform, and the milk inspector in the field.

From an educational standpoint, the present score cards present many problems. The lack of uniformity in the values allowed on the score cards for flavor when scoring milk, butter, cheese, or ice cream, is always difficult to explain, as well as the variation in the score which indicates the highest quality. If we are to hope for a greater interest in the quality of these products, we must furnish a simple understandable method of designating quality. It should be definite enough so that different judges can get similar results on the same product with the minimum amount of preliminary preparation. It would be very desirable if the score allowed for high quality could be the same for all dairy products. This would make it far easier for the layman or student to fix in his mind the levels at which high, medium, or low quality is expressed by numerical values.

With the question of simplicity as well as that of uniformity in mind, the writer has attempted to use the score grading system, which is followed in the *Handbook of Official United States Standards for Quality of Creamery Butter*,<sup>1</sup> for the scoring of milk, with the further thought in mind that cheese and ice cream might be likewise scored by the same system.

If this should be successful, much confusion could be avoided. Uniform high and low scores could be in the minds of the consumers as well as the

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\* The system proposed in this article is not sponsored by the American Dairy Science Association but is submitted in an attempt to stimulate interest in the subject and the possible unification of scoring methods for the four major products.—THE AUTHOR.

<sup>1</sup> U. S. D. A., Agricultural Marketing Service, February, 1940.

handlers of all dairy products. The use of terms could be made uniform and thus the whole problem of quality determination would be simplified.

The score grade method would allow the housewife to place a numerical value on a bottle of milk in the home. The milk grader on the factory platform could grade to a certain quality as designated by the numerical value. The question of bottle and sediment could be considered where it was possible. Its adaptation to a great variety of situations would seem to increase its value to all those interested in milk quality.

In an effort to meet these objectives, the Handbook of Official United States Standards for Quality of Creamery Butter have been followed as closely as possible in preparing a score grade method of scoring milk. With the completion of a similar system for ice cream and cheese, it is hoped that it may bring forth a practical, uniform, standard procedure for the determination of quality in the four major dairy products.

#### QUALITY OF MARKET MILK

The discontinuance of the factors of bacterial count, percentage of fat, acidity, and temperature will be noted. It may be assumed that the milk meets the requirements of the grade as designated on the package and that it meets the requirements of the public health authorities where it is offered for sale.

The standards for flavor provide ratings for different flavors and degrees of their intensity. Flavor and defects in bottle and cap and sediment are rated independently and the score is determined by application of a general rule as outlined. Only 9 points in a score range (85-93) are used, as is the case with official butter scoring.

#### *Section I—Terms Defined*

For the purpose of this discussion the grades here suggested refer to the quality of market milk.

(a) *Milk*—The lacteal secretion obtained from the complete milking of cows or goats.

(b) *Market Milk*—Milk which is used by the consuming public in a liquid form.

(c) *Score Grade*—The score grade of a lot of market milk consisting of packages of the same shall be expressed in terms of a score using whole numbers only. The score grades shall be from 85 to 93 inclusive.

#### *Section II—Grades for Market Milk*

The following grades for market milk are suggested.

(a) A 93 score will possess a fine, full flavor. It may not possess any noticeable defects in flavor. The total permitted defects in sediment are limited to a rating of one-half.

(b) A 92 score milk shall possess a pleasing flavor. It may possess a very slight normal feed flavor, be slightly flat, or have a very slight cooked flavor. The total permitted defects in sediment are limited to a rating of one-half unless the flavor rating is sufficiently high to permit the total ratings for defects in these factors to exceed one-half: provided, however, that the total ratings for defects in sediment must not exceed one in 92 score milk regardless of flavor rating.

(c) A 91 score milk shall possess a fairly pleasing flavor. It may possess a flat, slightly feed, or slightly cooked flavor. The total permitted defects in sediment and bottle cap are limited to a rating of one-half unless the flavor rating is sufficiently high to permit the total ratings for defects in these factors to exceed one-half.

(d) A 90 score milk shall possess a fairly pleasing flavor but may possess such flavors as definitely cooked, definitely feed, slightly salty, very slightly cowy, very slightly bitter, very slightly unclean, or very slightly oxidized. The total permitted defects in sediment and bottle and cap are limited to a rating of one-half unless the flavor rating is sufficiently high to permit the total ratings for defects in these factors to be in excess of one-half.

(e) An 89 score milk may possess any of the following flavors but only to a slight degree: salty, unclean, cowy, bitter, oxidized, and malty, or metallic or weedy to a very slight degree. The total permitted defects in sediment and bottle and cap are limited to a rating of one unless the flavor rating is sufficiently high to permit the total ratings for defects in these factors to exceed one.

(f) An 88 score milk may possess any of the following flavors to a definite degree: salty, unclean, cowy, bitter, oxidized, and malty, or metallic or weedy to a slight degree. The total permitted defects in sediment and bottle and cap are limited to one unless the flavor rating is sufficiently high to permit the total ratings for defects in these factors to exceed one.

(g) An 87 score milk may possess an onion or garlic flavor only to a slight degree. It may be definitely metallic or weedy. The total permitted defects in sediment and bottle and cap are limited to two unless the flavor rating is sufficiently high to permit the total ratings for defects in these factors to exceed two.

(h) An 86 score milk may possess a definite onion or garlic flavor or a pronounced metallic or weedy flavor. The total permitted defects in sediment and bottle and cap are limited to a rating of two unless the flavor rating is sufficiently high to permit the total ratings for defects in these factors to exceed two.

(i) An 85 score milk may possess a pronounced onion or garlic flavor or a very slightly rancid or high acid flavor. The total permitted defects in sediment and bottle and cap are limited to three unless the flavor rating is sufficiently high to permit the total rating for defects in these factors to exceed three.

(j) "No grade"<sup>2</sup> milk is milk that is below 85 score because of its flavor or other conditions or because of excessive defects in sediment and bottle and cap.

### *Section III—Basis for Determination of Quality of Market Milk*

The basis for determination of quality of market milk, except "no grade," shall be the ratings given flavor and the defects in sediment and bottle and cap. The score of individual samples of market milk shall be determined by the following general rule:

*General Rule:* The score of an individual sample of market milk shall be determined by deducting from the flavor rating<sup>3</sup> of the sample the amount that the total ratings of the defects in sediment and bottle and caps are in excess of the ratings for defects permitted in these factors for milk of the particular flavor rating (table 1), the score to be expressed as a whole number by lowering any half score to the next lower full score: provided, however, that the total ratings for defects in sediment must not exceed one in 92 score milk regardless of flavor rating.

### *Section IV—Ratings of Certain Identified Flavors in Market Milk*

The various identified flavors in market milk listed below shall be rated as follows:

<i>Identified Flavor</i>		<i>Flavor Rating</i>
(a)	Fine and full	93
(b)	Pleasing	92
(c)	Fairly pleasing	91-90
(d)	Normal feed	
	Very slight normal feed	92
	Slight normal feed	91
	Definite normal feed	90
(e)	Flat	
	Slightly flat	92
	Definitely flat	91
(f)	Cooked	
	Very slightly cooked	92
	Slightly cooked	91
	Definitely cooked	90
(g)	Salty	
	Very slightly salty	90
	Slightly salty	89
	Definitely salty	88
(h)	Cowy	
	Very slightly cowy	90
	Slightly cowy	89
	Definitely cowy	88

<sup>2</sup> See Section V for flavors that cause milk to be classified as "no grade."

<sup>3</sup> When more than one flavor is discernible in a sample of milk, the flavor rating for the sample shall be established by the flavor that carries the lowest rating.

(i) Bitter	
Very slightly bitter . . . . .	90
Slightly bitter . . . . .	89
Definitely bitter . . . . .	88
(j) Malty	
Very slightly malty . . . . .	90
Slightly malty . . . . .	89
Definitely malty . . . . .	88
(k) Oxidized	
Very slightly oxidized . . . . .	90
Slightly oxidized . . . . .	89
Definitely oxidized . . . . .	88
(l) Metallic	
Very slightly metallic . . . . .	89
Slightly metallic . . . . .	88
Definitely metallic . . . . .	87
(m) Weedy	
Very slightly weedy . . . . .	89
Slightly weedy . . . . .	88
Definitely weedy . . . . .	87
(n) Onion or garlic	
Slightly onion or garlic . . . . .	87
Definitely onion or garlic . . . . .	86
Pronounced onion or garlic . . . . .	85
(o) Rancid	
Very slightly rancid . . . . .	86
(p) High acid	
Very slightly high acid . . . . .	85
(q) Unclean	
Very slight unclean . . . . .	89
Slight unclean . . . . .	88
Definite unclean . . . . .	87

*Section V—Flavors and Conditions in Market Milk that Cause It  
To Be Classified as “No Grade”*

Market milk possessing the following flavors or in which the following conditions are present shall be classified as “no grade.”

A. <i>Flavors</i>	B. <i>Conditions</i>	C. <i>Bottle and Cap</i>
Rancid	Ropy or stringy	Dirty bottle
High acid	Bloody	(inside)
Chemical	Garget	Chipped lip
Pronounced un- clean	Clabbered	

*Section VI—Ratings for Defects in Sediment and Bottle and Cap*

**Rule (a):** Sediment as observed on disk shall show less than three very small specks.



<i>Defects</i>	<i>Rating</i>
Very slight (over 3 but under 10 very small specks) . . . . .	$\frac{1}{2}$
Slight (10 to 20 specks) . . . . .	1
Definite . . . . .	2

*Rule (b):* Clean bottle shall be full of milk, pouring lip protected by water-proof covering and sealed.

<i>Defect</i>	<i>Rating</i>
Not full . . . . .	$\frac{1}{2}$
Unprotected . . . . .	1
Partially protected . . . . .	$\frac{1}{2}$
Unsealed . . . . .	$\frac{1}{2}$
Leaky cap . . . . .	$\frac{1}{2}$

*Section VII—Defects Permitted in Sediment and Bottle and Cap without Causing Score To Be Placed Below Flavor Rating*

The maximum total ratings for defects in sediment and bottle and cap

TABLE 1

Flavor	Maximum total ratings for defects permitted in sediment and bottle and cap	Limitation of factor
93	$\frac{1}{2}$	1 factor (sediment only)
92	$\frac{1}{2}$	1 factor (sediment only)
91	$\frac{1}{2}$	1 factor only
90	$\frac{1}{2}$	
89	1	
88	1	
87	2	
86	2	
85	3	

TABLE 2

*Application of general rule*

Example No.	Flavor rating	Defects present in		Total defects present	Defects permitted	Defects in excess of those permitted	Final score
		Sediment	Bottles and cap				
1	93	0.5	0.0	0.5	0.5	0.0	93
2	93	0.0	1.0	1.0	0.5	0.5	92
3	93	0.5	1.0	1.5	0.5	1.0	91*
4	92	0.5	0.0	0.5	0.5	0.0	92
5	92	0.0	1.0	1.0	0.5	0.5	91
6	91	1.0	1.0	2.0	0.5	1.5	89
7	90	1.0	0.0	1.0	0.5	0.5	90
8	89	0.5	0.5	1.0	1.0	0.0	89
9	89	1.0	1.0	2.0	1.0	1.0	88
10	88	0.0	1.0	1.0	1.0	0.0	88
11	87	0.5	1.5	2.0	2.0	0.0	87
12	87	1.0	2.0	3.0	2.0	1.0	86
13	86	0.5	1.0	1.5	2.0	0.0	86
14	85	1.0	1.5	2.5	3.0	0.0	85
15	85	2.0	1.5	3.5	3.0	0.5	No grade

\* Score is lowered 2 points below instead of one because defects in 92 score milk must not exceed one point.

permitted in market milk that do not cause the score of market milk to be lowered below the flavor rating are given in table 1.

When the sum of the ratings for defects in sediment and bottle and cap exceeds that permitted by table 1 for market milk of a specified flavor rating, the market milk shall be given a score below the flavor rating in accordance with general rule (Section III) for determining the score of individual samples of market milk.

### *Section VIII—Application of General Rule*

In presenting this plan for determining the quality of milk, no attempt has been made to give at this time a discussion of details.

The factor of flavor and classification of flavors according to origin are very completely presented in the U. S. handbook on butter. The giving of the material here would only be a repetition. If the system is applicable to the four major products it would be possible to present a discussion of this nature which would furnish greater clarity and understanding of the terms used in describing the factors of quality in dairy products.

It is suggested that a copy of the *Handbook of Official United States Standards of Quality of Creamery Butter* be at hand if a critical study of the method is to be made. There would seem to be a large advantage in a uniform system for the four major products.



# THE REVERSIBILITY OF OXIDATIVE INACTIVATION OF MILK LIPASE IN RELATION TO ITS ACTIVITY IN CHEDDAR CHEESE

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The study of the properties of milk lipase in relation to the development of rancid flavor in cheddar cheese is a problem of some practical importance. In a recent publication the authors showed that a higher amount of proteolytic enzymes (rennet and pepsin) can bring about a decrease in the intensity of rancid flavor in cheddar cheese which contained added commercial lipase (9). Freeman and Dahle (7) showed a similar improvement in the flavor of cheddar cheese by the use of proteolytic enzymes without added lipase. Considering the presence of a small amount of endogenous milk lipase, their results may be explained on the same basis as ours. Other methods of inhibiting lipase activity in cheese remain to be investigated.

Copper in the presence of oxygen is an active lipase inhibitor. Davies (5) showed that the activity of milk lipase was depressed in unpasteurized butter to which 2–10 p.p.m. Cu had been added. Herrington and Krukovsky (8) confirmed this for raw milk. Later Krukovsky and Sharp (4) showed that dissolved copper caused no inactivation in the absence of oxygen. However, oxygen alone was active, its activity being greatly accelerated by small amounts of copper.

Oxidative inactivation of enzymes is common and, in general, reactivation is possible with reducing agents. Thus it is stated that the activity of serum lipase and human milk esterase is augmented proportionally with the degree of reduction (10). Our interest lies in the properties of milk lipase in raw milk cheddar cheese where strongly reducing conditions prevail (6). It was decided, therefore, to investigate whether oxidative inactivation of milk lipase was reversible in the presence of reducing systems simulating those in cheese.

Three systems were studied:

1. **Anaerobic**—Milk under anaerobic conditions develops a strongly negative potential. Aeration or copper cause the milk to maintain a high potential. The range of  $E_h$  thus covered is approximately  $-.2$  to  $.3$  volts. It may be expected, however, that the same systems would be operative in cheese where the interior is anaerobic.

2. **Ascorbic acid**—This acid is a normal constituent of milk and is held to be a part of an oxidation-reduction system concerned in preventing the

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development of oxidized flavor in milk (16). It is of further interest because ascorbic acid has been suggested as a component or a precursor of the coenzyme of lipase (11, 15). The oxidation-reduction potential of this system is reported as  $E_o' = -.066$  volts at pH 7 (4).

3. Cysteine—This amino acid was chosen because it is a good reductant. The oxidation-reduction potential of the cysteine-cystine system is given as  $E_o' = -.39$  volts at pH 7 (3). It may also be considered as a representative of the sulfhydryl system. It has been shown that ripened cheddar cheese gives a nitroprusside reaction characteristic of the  $-SH$  group (12). We have checked raw milk Canadian cheddar cheese and find the appearance at a very early stage of a positive nitroprusside test—the second or third day after manufacture. The sulfhydryl system is therefore not foreign to cheddar cheese. A general similarity of the reducing properties of sulfhydryl proteins or protein derivatives and amino acids might be expected.

#### EXPERIMENTAL

Two liters of raw whole milk were obtained from the Experimental Farm Dairy before the usual day's milk was pasteurized. The milk lipase in the sample was then activated by the method of Krukovsky and Herrington (12). The milk was pre-cooled in ice water and subsequently warmed to 30° C. At this stage it was divided, as required, into 3 or 4 portions of 500 ml. each and placed into wide-mouth bottles of 1 liter capacity. The bottles were fitted with large 2-hole rubber stoppers and glass tubing so that the milk could be aerated by aspiration using a water pump or deaerated by nitrogen gas from a cylinder. Each bottle of milk was then treated according to plan.

As an example of the treatment, let us consider experiment no. 12, table 3. From a stock solution of  $CuCl_2$  2 p.p.m. Cu was added to each bottle of milk. Nitrogen was bubbled through the first bottle for 15 minutes. It was then aerated for the remaining 30 minutes. To the second bottle of milk was added 100 mg. of Merck's cysteine hydrochloride dissolved in a small quantity of water. (100 mg. of Merck's l-ascorbic acid was used in experiments 6-9, table 2.) It was aerated for 30 minutes and then deaerated by bubbling nitrogen for an additional 15 minutes. The third bottle was treated in the same way, except that cysteine was added after aeration was completed. The last bottle was deaerated for 45 minutes. In this way each portion of milk received a total of 45 minutes of agitation by bubbling in order to avoid unequal activation of milk lipase by this method (13). Also bottles 1-3 received the same amount of aeration. At the end of this treatment the first bottle was allowed to remain open to the air, while in the remaining bottles both the inlet and the exit tubes were clamped. These bottles were then stored in a refrigerator at 5° C.

On the fourth day the milk lipase activity was determined. The rubber

stoppers were replaced with ground glass stoppers, the milk was warmed to 17° C. and churned in an end-over-end shaker. The butter granules so obtained were filtered off on a Buchner funnel using a cheese cloth filter. The butter was then transferred to test tubes, melted in a 60° C. oven and the butterfat pipetted off into 15-ml. centrifuge tubes. The centrifuge tubes were warmed and centrifuged to separate the final traces of the aqueous phase. Three to five gm. portions of the clear butterfat in 50 ml. of 95 per cent boiling ethanol were titrated in duplicate using N/20 NaOH and phenolphthalein indicator. Since we were interested only in the differences in lipase activity of the variously treated milk the alcohol was used without neutralization. The values are recorded as ml. of N NaOH per 100 gm. butterfat. The results are shown in tables 1 to 3.

TABLE 1

*The effect of aeration, deaeration and copper on milk lipase*

Expt. No.	Cu	Aerated	Aer.-deaerated	Deaerated
	<i>p.p.m.</i>			
1	0	3.1	3.9	4.4
2	1	1.8	1.7	2.6
3	2	2.1	2.1	3.1
4	4	1.3	1.3	2.3
5	8	1.0	1.1	2.1

TABLE 2

*The effect of aeration, deaeration, copper and ascorbic acid on milk lipase*

Expt. No.	Cu	Aerated	Asc. acid aer.-deaerated	Aer.-asc. ac.-deaerated	Deaerated
	<i>p.p.m.</i>				
6	0	1.9	2.4	2.1	2.6
7	0	1.4	1.6	2.2	2.6
8	2	0.9	0.7	0.7	1.7
9	2	2.3	1.8	2.3	3.1

TABLE 3

*The effect of aeration, deaeration, copper and cysteine on milk lipase*

Expt. No.	Cu	Aerated	Cysteine-aer.-deaerated	Aer.-cysteine deaerated	Deaerated
	<i>p.p.m.</i>				
10	0	1.8	2.8	2.9	2.5
11	2	1.0	2.0	1.8	1.5
12	2	1.3	3.3	2.5	2.2

All values expressed as ml. N NaOH per 100 gm. butterfat.

#### DISCUSSION

From table 1 it may be seen that in milk to which 1-8 p.p.m. Cu was added there was no significant difference between the lipase activity of the

corresponding aerated and the aerated-deaerated samples while in each case the deaerated sample showed a higher lipase activity. This indicates that deaeration of milk under the conditions described does protect lipase from oxidation, confirming the results of Krukovsky and Sharp. However, the deaeration of aerated milk gave no protection when copper was present. In the milk containing no added copper the lipase activity of the aerated-deaerated milk was between that of the aerated and the deaerated samples. This indicates that some of the inactivated lipase was probably reduced back to its original active state under anaerobic conditions. This view is confirmed by the cysteine experiments.

The experiments with ascorbic acid (table 2) gave erratic results. Either the ascorbic acid cannot reduce oxidized lipase or it is too readily destroyed to be an effective reducing agent under our experimental conditions.

Cysteine yielded the most interesting results. It will be seen from table 3 that, with or without the addition of copper, whether cysteine was added before or after aeration, the milk lipase activity was highest in the aerated-deaerated milk. The following conclusions might be drawn. In the cysteine-aerated-deaerated milk the lipase was either protected against oxidation or the oxidized milk lipase was subsequently reduced by the cysteine present. In the aerated-cysteine-deaerated milk the latter mechanism is supported, *i.e.*, the reversibility of oxidatively inactivated lipase is indicated rather than straightforward protection. Finally, since the cysteine experiments showed higher lipase activity than even the deaerated milk augmentation of lipase is indicated. This might be interpreted as the reduction of milk lipase which was originally in the oxidized or inactive form in the milk. Or, some destruction of lipase occurred in the deaerated milk due to a small amount of oxygen or other oxidizing substances.

From these data it might be inferred that any oxidized milk lipase would be reduced in cheddar cheese, thus restoring it to its active condition. Oxidative inactivation as a means of lipase inhibition would therefore be limited to the possible proteolytic inactivation of oxidized lipase before reduction took place. Experimental cheese work is being done to study this phase of the investigation.

#### SUMMARY

The reversibility of oxidative inactivation of milk lipase has been studied using three oxidation-reduction systems of interest in cheddar cheese. It is concluded that cysteine can reverse the inactivation of milk lipase brought about by aeration, or by aeration and copper. Some augmentation of lipase activity has also been noted. Anaerobic environment may bring about some reversibility of oxidatively inactivated milk lipase, but not in the presence of copper. The results with ascorbic acid are inconclusive.

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# VARIOUS OILS AND FATS AS SUBSTITUTES FOR BUTTERFAT IN THE RATION OF YOUNG CALVES<sup>1</sup>

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The literature dealing with the value of various fats and oils in nutrition is very extensive, yet but few studies have been reported comparing their feeding value with butterfat for young calves. This fact is rather unusual considering the obvious practical value of such information in relation to economical calf raising and especially to profitable veal production.

The studies reported have largely been confined to a comparatively few oils and fats and results have generally been unfavorable. Lindsey (7) using calves several months old reported generally favorable results from feeding "oleo" at the rate of one ounce per quart of skim milk and mixed thoroughly. He states, "Scarcely any of the calves were able to take more than this amount per quart without disturbing their digestion." Other products used were corn oil and cottonseed oil, but these could be fed only at the rate of one-half ounce per quart of skim milk "without producing bad effects." A calf fed a combination of corn oil and cottonseed oil seemed to thrive at first but later its condition became less favorable and when slaughtered the carcass "contained very little fat." Hendricks (5), also reported less growth in calves fed cottonseed oil and skim milk than in those fed whole milk or skim milk and cod liver oil. Leach and Golding (6) using calves 15 to 22 days old fed pilchard oil homogenized into skim milk. Severe scouring developed but increases in weight continued during the first week, then their condition became unsatisfactory and in no case did a calf live more than three weeks. Rats on a similar diet grew satisfactorily and produced litters of living young. Schmalfuss and co-workers (11) found emulsified coconut oil to be equal to cod liver oil for feeding to calves. Similarly, Fingerling (3) found that emulsified peanut oil was a satisfactory supplement to skim milk for calves provided it was not added in too great amounts.

In 1939 we (4) reported, very briefly, our results obtained from feeding calves butter oil, lard, corn oil, cottonseed oil and soybean oil, respectively, homogenized into skim milk. The results as measured in terms of rate of gain in weight, physical appearance and general well-being of calves indicated clearly the superior nutritive value of butterfat over all the other fats and oils tested. The calves fed lard made nearly as rapid gains in weight

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but were inferior in appearance to those receiving butterfat. The animals fed the other three oils made little or no gains in weight and were very poor in appearance and some of them died. Evidence indicating similar differences in the nutritive value of these fats and oils for rats has more recently been presented by Schantz, Elvehjem and Hart (8).

Because of the nature of the results obtained in our original experiments, the study has been continued and enlarged. Some of the earlier work has also been repeated. The present paper is a report on the work completed to date.

#### EXPERIMENTAL

The following oils and fats were tested in feeding trials: soybean oil, corn oil (Amaizo and Mazola), cottonseed oil (Wesson), coconut oil, peanut oil, lard, beef tallow and butterfat. The latter was prepared by oiling off freshly churned unsalted butter from unpasteurized cream. As in our previous trials, the feeding value of different oils and fats for young dairy calves was measured by rate of gain in weight or growth, physical appearance and general well-being of animals.

The calves used were grades, crossbreds and purebreds of the various dairy breeds and included both males and females obtained from several dairy herds, including the one at University Farm. Calves were usually left with their dams for several days after birth or until they had received colostrum milk. They were then taught to drink from a pail and put on the experimental diet at a week to two weeks of age, and in some cases later. Environmental conditions were kept as nearly uniform as possible for all animals but not all trials were run concurrently. All animals included were adjudged healthy at the time they were placed on experiment.

The fats or oils, except as indicated, were mixed at the rate of 3.5 pounds to every 96.5 pounds skim milk and emulsified at a temperature of about 120° Fahrenheit by means of a Gaulin type homogenizer at 3000 pounds pressure, single action. Each mixture was usually run through the machine three times to insure more complete homogenization. The resulting product was fed at a temperature of 90° Fahrenheit and at the rate of one pound per day per 10 pounds of live weight of calf, except as otherwise indicated. In order to check on any possible effect of the process of homogenization on the butter oil in skim milk, several calves were fed normal whole milk. Hay (alfalfa) and concentrates were usually not fed until the calf was about a month old. In some cases no alfalfa hay was fed. The concentrates mixture was designed to be very low in fat. It was made up of 200 pounds ground dry beet pulp, 50 pounds dry skim milk and 50 pounds gluten meal.

To determine whether calves require any fat in their diet a very fat-poor diet was fed to one group of calves. It consisted of skim milk (.01-.02 per cent fat) along with a concentrates mixture of 100 pounds ground molasses

beet pulp, 50 pounds dry skim milk, 50 pounds starch and 25 pounds cerelese. No hay was fed to calves in this group.

Each calf in all groups was fed 25 to 35 cc. U.S.P. cod liver oil daily or its equivalent in concentrated products. The plan was for all calves to receive the same amounts of nutrients according to weight and at a level adequate for growth, but this was found to be impossible in some cases because of inability or lack of desire on the part of the animal to consume the required amount of food.

Each calf was kept in a separate pen, fed individually and regularly twice daily, all feed was carefully weighed or measured and amounts consumed recorded. All calves were turned outdoors for exercise several hours daily when weather permitted. Weights of each animal were obtained regularly, some at weekly intervals and others every ten days. Frequent observations were made and recorded in regard to physical condition and other facts about each animal. Post mortem examination was made of all animals that were slaughtered or died.

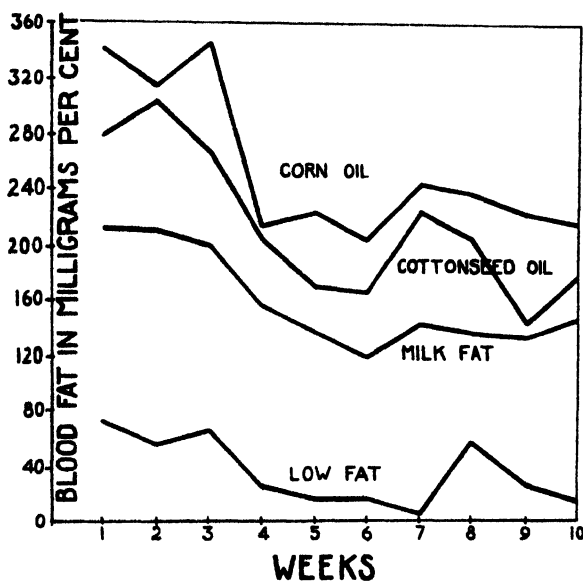


FIG. 1. Average fat content of blood of various groups. Age of animal not considered.

Blood samples for plasma fat volume determination by Allen's method (1) were obtained at weekly intervals from a few animals after they were 90 days old. These data are presented graphically in figure 1. Attempts were also made with several calves about four months old to determine the course of the absorbed fat in the body by means of fat stains or dyes, but results invariably were negative.

## RESULTS

No real difficulty was encountered in getting calves to drink the desired amounts of the various prepared skim milk, oil or fat products. However,

TABLE 1

*Average weight at 30-day intervals and nutrient per day and per 100 pounds live weight of calves fed various oils and fats*

Age days	Weight lbs.	Nutrients per day		T.D.N. per* 100 lbs. weight	Weight lbs.	Nutrients per day		T.D.N. per* 100 lbs. weight
		Protein	T.D.N.			Protein	T.D.N.	
		lbs.	lbs.	lbs.		lbs.	lbs.	lbs.
Whole Milk Group (2 calves)					Butter Oil Group (2 calves)			
30	100	.35	1.73	1.96	108	.35	1.70	1.72
60	140	.38	1.89	1.58	141	.45	2.19	1.75
90	186	.45	2.37	1.45	176	.67	3.23	2.03
120	231	.59	3.14	1.50	225	.74	3.58	1.79
	Average			1.62				1.82
Low Fat Group (5 calves)					Lard Group (6 calves)			
30	101	.31	1.16	1.18	104	.30	1.45	1.54
60	123	.48	1.53	1.37	135	.49	2.36	1.98
90	155	.73	2.60	1.87	163	.57	2.79	1.87
120	176	.88	3.07	1.85	209	.73	3.55	1.91
	Average			1.57				1.83
Tallow Group (4 calves)					Coconut Oil Group (4 calves)			
30	89	.27	1.33	1.64	86	.24	1.19	1.55
60	119	.41	2.02	1.94	112	.30	1.46	1.47
90	165	.71	3.45	2.43	141	.48	2.32	1.84
120					176	.65	3.14	1.99
	Average			2.00				1.71
Peanut Oil Group (3 calves)					Corn Oil Group (6 calves)			
30	84	.29	1.43	1.83	79	.29	1.42	1.82
60	112	.40	1.94	1.98	92	.32	1.48	1.74
90					109	.38	1.72	1.72
120					123	.38	1.79	1.54
	Average			1.90				1.71
Cottonseed Oil Group (2 calves)					Soybean Oil Group (5 calves)			
30	85	.29	1.42	1.73	87	.25	1.12	1.32
60	104	.31	1.30	1.38	102	.30	1.29	1.37
90	119	.36	1.51	1.36	109	.36	1.52	1.45
120	127	.49	2.31	1.88	132	.39	1.62	1.35
	Average			1.59				1.37

\* Based on average weight during period.

it was necessary in some cases, due to poor physical condition of the calf, to either reduce the amount of the product fed, to change temporarily to whole milk, or to reduce the fat content of the milk fed (see table 2). This occurred almost wholly with calves fed either corn oil, cottonseed oil or soybean oil. The necessity of limiting the food intake in these groups made equivalent reductions necessary in other groups in order to keep them on approximately the same nutrient intake basis. The average nutrient intake of the various groups at different ages and weights is indicated in table 1. Table 2 indicates the kind of fat or oil fed and the fat content of milk fed to each calf along with facts relating to its physical condition. It also shows the age and weight of the animal at start and end of experimental period, together with average daily gain in weight of each group. Figure 2 indicates the growth of the calves in each group.

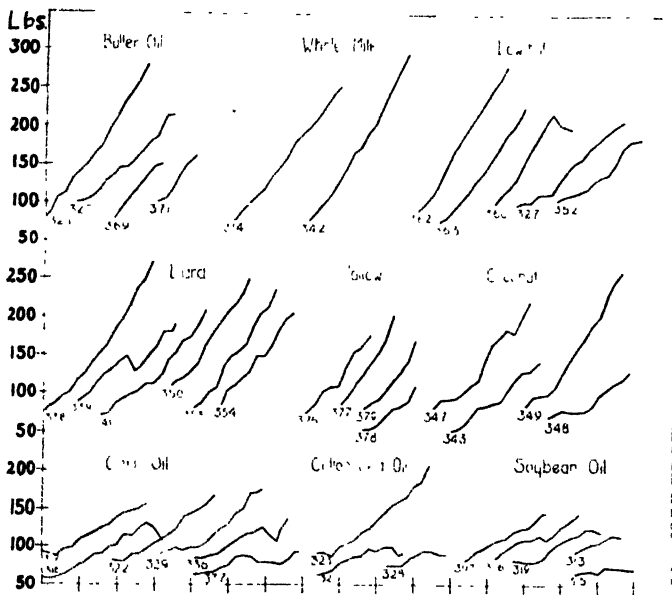


FIG. 2. Growth curves of calves fed various fats and oils.

It will be observed from table 2, that the calves fed fats of animal origin, butterfat, tallow and lard, made significantly greater average daily gains in weight than those receiving the vegetable oils, but especially soybean oil, cottonseed oil and corn oil. Another significant difference noted between the groups was the greater amount of fat present in the carcasses of milk-fat fed calves. Even lard- and tallow-fat fed calves that had made good gains in weight and were in healthy, thrifty condition when slaughtered were inferior in this respect. This fact may be of special significance in relation to the quality of veal produced.

No apparent differences were observed between calves fed whole milk and those receiving the butter oil homogenized in skim milk. Both groups were

TABLE 2

*Number, breed and sex of calves fed different fats and oils; their age and weight at beginning and end of experimental period and physical condition while on experiment; also, fat content of milk fed*

Number, breed & sex of calf			Age—days		Weight—lbs.		Physical condition of calf, fat content of milk fed, etc.	
			Start	End	Start	End		
Whole Milk Group								
314	Gr. H.	M	7	147	77	253	3.5 per cent fat.	Condition excellent.
342	" "	F	7	149	75	301	" " " "	" "
Average daily gain in weight, 1.43 pounds								
Butter Oil Group								
324	Gr. H.	M	10	141	84	284	3.5 per cent fat.	Condition excellent.
325	" Ayr.	"	8	137	98	209	" " " "	" "
369	" H.	"	9	65	80	150	" " " "	" "
371	" "	"	9	60	100	160	" " " "	" "
Average daily gain in weight, 1.22 pounds								
Low Fat Group								
327	Gr. H.	M	9	154	100	211	Indigestion at start, otherwise thrifty and healthy.	
352	" "	"	11	126	106	185	Severe indigestion part of time; condition fair.	
360	" "	"	22	128	100	214	Fed up to 18 pounds skim milk daily; condition excellent.	
362	" "	F	22	140	92	280	Fed up to 18 pounds skim milk daily; condition excellent.	
363	Jer.	M	20	135	76	227	Fed up to 18 pounds skim milk daily; condition excellent.	
Average daily gain in weight, 1.07 pounds								
Lard Group								
338	Gr. H.	M	6	150	75	270	3.5 per cent fat. Condition good.	
339	" "	"	6	148	89	205	3.5 per cent fat-lard milk to 82 days of age. Scoured, weak. Whole milk to 106 days of age. Scouring ceased. 3.5 per cent lard-milk to end. Condition fair to good.	
340	Guern.	M	7	151	71	212	3.5 per cent lard milk. Scoured occasionally. Condition good.	
350	Gr. H.	F	15	119	110	250	3.5 per cent lard milk. Scoured occasionally. Condition good.	
353	" "	M	12	122	82	238	3.5 per cent lard milk. Scoured occasionally. Condition good.	
354	" "	F	17	119	85	207	3.5 per cent lard milk. Scoured occasionally. Condition good.	
Average daily gain in weight, 1.17 pounds								
Tallow Group								
376	Gr. H.	F	15	105	75	182	3.5 per cent tallow milk fed up to 18 lbs. daily. Some scouring but condition fair to good.	
377	" "	M	8	81	88	206	3.5 per cent tallow milk fed up to 18 lbs. daily. Some scouring but condition fair to good.	

TABLE 2—(Continued)

Number, breed & sex of calf		Age—days		Weight—lbs.		Physical condition of calf, fat content of milk fed, etc.	
		Start	End	Start	End		
Tallow Group (Continued)							
378	Jer.	M	12	85	53	118	3.5 per cent tallow milk. Some scouring but condition fair to good.
379	Gr. H.	“	12	83	80	170	3.5 per cent tallow milk. Some scouring but condition fair to good.
Average daily gain in weight, 1.24 pounds							
Coconut Oil Group							
343	Jer.	M	14	134	52	143	3.5 per cent oil-milk. Indigestion occurred but condition fair to good.
347	Gr. H.	M	12	151	81	233	3.5 per cent oil to age 31 days. Indigestion. Whole milk to 39 days, then 3.5 per cent oil to end. Condition fair to good. Fair amount of internal fat present.
348	Gr. H.	F	12	120	71	131	3.5 per cent oil-milk fed. Scoured. Condition poor to fair.
349	“ “	M	9	140	85	260	3.5 per cent oil-milk fed. Scoured. Condition poor to fair.
Average daily gain in weight, .96 pounds							
Peanut Oil Group							
381	Jer.	M	11	92	62	125	3.5 per cent oil-milk. Scoured some but condition fair to good.
382	Guern.	M	12	63	65	100	3.5 per cent oil-milk. Scoured some but condition fair to good.
383	Gr. H.	M	9	63	90	141	3.5 per cent oil-milk. Scoured some but condition fair to good.
Average daily gain in weight, .80 pounds							
Corn Oil Group							
316	Guern.	F	12	170	59	107	3.5 per cent oil to age 21 days. Scoured and dermatitis. Fed half and half whole milk and corn oil milk to age 32 days, then whole milk to 41 days, 2 per cent corn oil milk to 105 days and 3.5 per cent oil to end. Declined gradually in strength and died. Emaciated, with little or no depot fat. Liver pale and friable, kidneys showed fatty degeneration.
317	Gr. H.	M	12	148	92	155	3.5 per cent oil to age 31 days. Scoured. Whole milk to 41 days. Scours ceased. 2 per cent oil to 106 days. No indigestion, then 3.5 per cent oil to end. Gained slowly. Appeared unthrifty.
322	Gr. G.	F	14	151	83	168	3.5 per cent oil to age 34 days. Indigestion and dermatitis. Whole milk to 44 days. 2.0 per cent oil to 139 days of age then 3.5 per cent oil. Fairly thrifty when discontinued.



TABLE 2—(Continued)

Number, breed & sex of calf	Age—days		Weight—lbs.		Physical condition of calf, fat content of milk fed, etc.
	Start	End	Start	End	

Corn Oil Group (Continued)							
329	Guern.	M	9	154	87	176	3.5 per cent oil throughout but limited to not over 10 pounds milk daily. Severe dermatitis and slow gain in weight. Appeared emaciated and unthrifty.
336	Guern.	M	21	150	86	137	3.5 per cent oil milk fed. Slight indigestion. Very slow or no gain in weight. Thin and rough appearance.
337	Guern.	M	11	155	64	95	3.5 per cent oil-milk fed but limited to not over 7 lbs. daily. Scoured. Some dermatitis. Little or no gain in weight. Weak and emaciated. Died.
385	Albino	F	13	33	56	40	3.5 per cent oil-milk fed. Severe scouring. Died. Hock joints swollen. Severe gastritis and enteritis.
392	Jer.	M	10	61	59	75	3.5 per cent oil-milk fed. Slight indigestion. Was quite active and playful. Good appetite but thin. Hair rough. Became weak and unable to stand. When changed to whole milk at 61 days made rapid recovery.

Average daily gain in weight, 40 pounds

Cottonseed Oil Group							
321	Gr. H.	F	12	126	65	92	3.5 per cent oil to age 29 days. Unthrifty and slight indigestion, weak. Whole milk to 41 days, 2 per cent oil milk to 103 days of age, 3.5 per cent oil to 124 days, then whole milk again. Thin, emaciated appearance, rough, weak, died. Little or no internal fat. No evident changes in liver and kidneys.
323	Gr. H.	M	10	151	93	209	3.5 per cent oil to age 27 days. Whole milk to 39 days, 2 per cent oil to 100 days, then 3.5 per cent oil milk. This was a very rugged calf at start. Little or no scouring, considerable loss of hair. Ate considerable alfalfa hay. Gained slowly but steadily. Appearance fair.
328	Gr. H.	F	10	85	77	90	3.5 per cent oil milk throughout. No indigestion. General decline set in at about 8 weeks of age. Died. Several hemorrhagic areas in abomasum. Decided absence of depot fat.
389	Gr. H.	M	6	45	85	77	3.5 per cent oil milk throughout. Weakened, declined, died. Emaciated appearance.

Average daily gain in weight, 31 pounds

Soybean Oil Group							
305	Gr. H.	M	7	39	90	87	3.5 per cent oil milk. Severe indigestion. Died.

TABLE 2—(Continued)

Number, breed & sex of calf			Age—days		Weight—lbs.		Physical condition of calf, fat content of milk fed, etc.
			Start	End	Start	End	
Soybean Oil Group (Continued)							
306	Gr. H.	F	29	144	87	144	2.0 per cent oil milk limited to not over 10 pounds daily. Rough and unthrifty throughout.
307	" "	"	13	125	83	144	3.5 per cent oil milk to age 25 days. Scouring. Then 2 per cent oil milk limited to not over 10 pounds daily. Unthrifty appearance. Condition poor.
313	Gr. H.	M	10	93	96	121	3.5 per cent oil milk to age 29 days. Slight indigestion. Whole milk to 41 days, then 2 per cent oil milk. Thin, rough, slow gain in weight. Declined and died. Decided lack of depot fat. No other significant abnormalities noted.
315	Gr. H.	F	11	95	65	72	3.5 per cent oil milk to age 30 days—refused to drink it. Whole milk to 42 days, then 2 per cent oil milk. Some loss of hair. No indigestion. Thin and scrawny. Declined in strength. Unable to stand. Heart was flaccid and edematous. Evidence of gelatinous infiltration in kidneys. No evident internal fat.
319	Gr. H.	M	20	129	83	120	3.5 per cent oil milk to age 40 days. Scoured with considerable loss of hair. Whole milk to 52 days. Scouring ceased. 2 per cent oil milk to 115 days, then 3.5 per cent oil to 129 days. Became weak and unable to stand; when changed to whole milk made remarkable recovery.
386	Jer.	M	14	26	52	44	3.5 per cent oil milk fed. Became weak and died.
Average daily gain in weight, .32 pounds							

healthy and thrifty with sleek coats and bright eyes. Although the lard- and tallow-fed groups made almost, if not, as rapid gains in weight as those receiving the milk fat, they were slightly inferior in general appearance but were nevertheless thrifty and alert.

The excellent gains made by calves on the low-fat diet (average daily gain 1.07 pounds) perhaps suggest that they have no need for more than the extremely limited amount of fat provided in their ration. In appearance the calves in this group were typical of calves raised on skim milk under farm conditions. They were healthy and thrifty but did not have quite the sleek, well-fed appearance of calves raised on whole milk. It should be added that several of the animals in this group were continued on the low-fat diet until about two years of age. They remained normal in appearance and made excellent gains in weight throughout.

The condition of the calves in the groups fed coconut oil and peanut oil respectively were on the whole inferior to calves fed lard or tallow but were definitely superior to the animals fed either corn oil, cottonseed oil or soybean oil. In the latter three groups, the calves almost invariably appeared thin and emaciated with rough unkempt hair. Some of them also showed a characteristic loss of hair or dermatitis, the areas about the face, ears and neck being first affected. Subsequent losses occurred on the lateral and medial areas of the cannons of the rear legs. A brown, oily-like crust covered the denuded areas. The time of the appearance of the condition, its extent and duration varied widely in different individuals. The fact that some animals in these groups were not affected, that it appeared in a few individuals in other groups and also that it sometimes occurs in calves on normal rations makes it difficult to suggest a probable cause.

Indigestion or scours appeared among the calves in all groups but those fed corn oil, soybean oil and cottonseed oil were the most seriously affected. Some calves in these groups died from this disorder at an early age (data not included) and others probably would have done so if the ration had not been changed as indicated in table 2. Others in these three groups, although not affected by scours, gained very slowly in weight for a time although they appeared rather haggard and dull, as though starving. This was followed by gradual weakening and some loss in weight, often terminating in death if whole milk was not substituted in time. Several calves (319 and 392) that were in a very weakened condition and unable to stand made remarkable recoveries after such a change in diet was made.

#### DISCUSSION

The study indicates that under the conditions of the trials, butterfat was superior to all other fats and oils tested as a food for young dairy calves. It appears that tallow and lard may also be used quite satisfactorily for this purpose under the plan of feeding followed. The reason for the superiority of milk fat over other fats and oils tested and for the reasonably good results obtained with the lard and tallow is not indicated by the data. It is true that the calves in these groups were fed on a slightly higher plane than those in some of the other groups but this alone is hardly sufficient to account for the marked differences observed in the rate of gain in weight and physical condition of the calf. No doubt the more frequent and perhaps more severe cases of scours among calves in the corn oil, cottonseed oil and soybean oil groups affected the results but this was probably not the most important factor involved, for death or slow gain in weight also occurred in calves in these groups in which indigestion was absent. It may be pointed out as a matter of general observation in regard to these three groups that the older the calf and the more vigorous it was when placed on experiment the better the results obtained. The latter is probably the chief reason for the fairly satisfactory growth of calf No. 323 on cottonseed oil.

A question may be raised as to whether or not each of the various oils actually were digested and absorbed into the body. Unfortunately, no digestion trials were conducted. However, the relatively high fat volume of the blood plasma of the animals fed the less satisfactory oils as compared to that of those fed milk fat suggests that these oils probably were absorbed. Too much emphasis should not be given to this fact, however, because of the very limited amount of data on hand. What happened to these oils if they actually were absorbed is not known. Were they altered and later excreted through the skin, causing the dermatitis-like condition previously described, or were they excreted back into the intestine? Only further investigations can answer these questions. Post mortem examinations indicated that they were not stored as depot fat in the body to any great extent.

No attempt will be made at this time to explain the differences noted in the nutritive value of the fats and oils tested. It may be pointed out, however, that the work of Burr and Burr (2) and the more recent studies of Hart and co-workers (9, 10) suggests that the nature and kind of fatty acid combinations present may be extremely important. Also, it is possible that some of the oils fed lacked in certain essential factors or that they contained substances toxic to the young calf. We are now investigating these and other phases of this problem.

#### SUMMARY

Feeding tests were conducted to compare the feeding value of the following fats and oils for calves: butterfat, lard, tallow, coconut oil, peanut oil, corn oil, cottonseed oil and soybean oil. The effect of a very fat-poor diet on calves was also determined. Each oil or fat was added to skim milk, homogenized to form a product containing 3.5 per cent fat and fed along with a low fat content concentrate mixture, cod liver oil and some alfalfa hay. One control group was fed normal whole milk not homogenized. Test periods ranged from a few days to about six months.

In average daily gain in weight as well as in general well-being, the calves fed butterfat excelled those in all other groups. Following closely were those receiving lard and tallow. Corn oil, cottonseed oil and soybean oil were the least satisfactory. The average daily gains of calves in the latter three groups were .40 pound, .31 pound and .32 pound, respectively. They appeared unthrifty, listless and emaciated. Some calves in these groups died and others were saved only by changing to whole milk.

Post mortem examinations showed considerably more fat deposited in calves fed butterfat than in those that had been fed other oils and fats.

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# THE INTRODUCTION OF CATTLE INTO COLONIAL NORTH AMERICA\*

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The scarcity of data relative to the first importations of cattle into Colonial North America has lent obscurity to one of the most interesting phases of early American husbandry. In fact this paucity and incompleteness of information dealing with the introduction of cattle into what is now the United States of America has led many authors in the field of animal and dairy husbandry to an almost studied disregard of this primary stage in the development of our national livestock industry. When it is considered that the foundations of cattle husbandry were laid in every one of the thirteen original colonies and in the south and southwestern part of our present United States before any appreciable progress had been made in the systematic improvement of cattle in England and Continental Europe, the question of where our foundation animals came from should be of more than passing interest. Allen (2) in 1890 in his work, **American Cattle**, dealt briefly with colonial cattle importations. Bidwell and Falconer (6) in 1925 and Gray (25) in 1933 in their general histories of agriculture in the United States to 1860 have presented a considerable amount of information relative to early importations. It was with the hope of assembling, in one body, additional information on the introduction of cattle into Colonial North America that this review of literature was undertaken.

It is quite apparent that Colonial Americans were so busy making economic history that they failed to write sufficiently about it. Authentic records were, in many cases, incidental and are found in several fields. This has increased the difficulty of presenting data that are both complete and accurate. Quotations have been offered frequently in order that a better picture might be had of the actual conditions and circumstances surrounding many of the colonial cattle raising undertakings. It is hardly necessary to point out that this review of literature is incomplete. It is offered at this time, however, with the thought that it may be of some assistance to the teachers of courses in dairy cattle history.

During the period of discovery and colonization there were four possible paths of introduction of cattle into what is now the United States of America. First, from the West Indies to any portion of the Atlantic and Gulf of Mexico coast line. Second, from Mexico into southwestern areas and California. Third, from the French colonies of the St. Lawrence Valley into

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the area of the "Old Northwest." Fourth, directly from the colonizing European nations to the American colonies. Historical evidence supports the belief that all of these paths of entry were used.

#### INTRODUCTION OF CATTLE BY THE SPANISH

Encouraged by the glowing reports carried back to the Old World by Columbus in 1492, the Spanish throne decided to colonize the New World at once. To this purpose Columbus made a second voyage in 1493, carrying with him besides colonists, a large variety of agricultural seeds and plants, and the first shipment of domestic livestock destined to inhabit the New World. "Besides a few horses for cavalry service there were carried for breeding purposes mares, sheep, heifers and other animals. Vegetables, wheat, barley and other cereals were not forgotten nor the vine and fruit trees. All kinds of tools, too, that would be needed in a colony were included. At the Canary Islands they added to their stock calves, she-goats, ewes, pigs, chickens, seeds of oranges, lemons, and other garden plants, and most of all, sugar cane" (37). It was necessary for Columbus to carry these things to the new lands for, though "gloriously rich in some aspects of nature, the New World was notably poor in food plants and domestic animals" (8).

Whether the attempts to develop an animal industry in the New World were at once successful is not known. Because of the small size of sea-going ships during the sixteenth century, it is entirely possible that only a small number of cattle, or more correctly "neat cattle," were brought over at first. As the first shipment was made up of heifers and calves, it can be concluded that for the first few years there could not have been a large number of cattle for slaughter. Although we have no information that would lead one to believe the Spanish were large consumers of beef or dairy products, yet there was an immediate need for cattle above the numbers taken over, and their increase. In an attempt to fill this need, Columbus, in 1494, urged the Spanish King and Queen to authorize contractors to deliver to the new country cattle and beasts of burden annually, for which they might be paid by giving them Indian slaves (53). Whether or not this recommendation of Columbus' was adopted and followed to any great extent, we do not know. By 1512, however, stock-raising had become a fixed industry in the West Indies (8), and considerable numbers of cattle were being raised.

The Spanish took cattle from one island to another in the West Indies until they became quite common in the eyes of voyagers who had occasion to stop from time to time. Hakluyt (28) in relating the voyage of John Hawkins to the West Indies in 1565 said "The tenth day (of March) at night we departed from thence (the first island seen) and the fifteenth had sight of nine islands, . . . and the sixteenth of an island, called Margarita,

where wee were entertayned by the alcalde, and had both Beeves and sheepe given us. . . ." During this same voyage "The sixth of May aforesaide, we came to an yland called Curacao. . . . In this place we had trafique for hides, and found great refreshing both of beefe, mutton and lambs, where of there was such plentie, that saving the skinnes, we had the flesh given us for nothing. . . ."

"The increase of cattell in this yland is marveilous, which from a doozen of each sort brought thither by the governour, in 25 years he had a hundreth thousand at the least & of other cattel was able to kill without spoile of the increase 1500 yeerely, which he killeth for the skinnes, and of the flesh saveth only the tongues, the rest hee leaveth to the foule to devoure. And this I am able to affirme, not onely upon the Governours own report, who was first to bring the increase thither, which so remaineth unto this day, but also by that I saw my selfe in one field, where an hundred oxen lay one by another all whole, saving the skinne and tongue taken away. And it is not so marveilous a thing why they doe thus cast away the flesh in all the ylands of the West Indies, seeing the land is great, . . . the people fewe, having delicate frutes and meates ynough besides to feede upon, which they rather desire, . . . : for in S. Domingo an yland called by the finders thereof Hispaniola, is so great a quantitie of cattel, and such increase thereof that notwithstanding the daily killing of them for their hides, it is not possible to asswage the number of them, but they are devoured by wilde dogs, . . . that they eate and destroy 60,000 a yeere, and yet small lacke found of them."

These quotations give the impression that the cattle taken by the Spanish adventurers into the New World possessions were primarily for the purpose of furnishing hides, with beef tongues as a secondary consideration, or what might be termed a by-product of the hide-producing industry. In fact, hides seem to have been one of the leading export articles, not only of the West Indian Islands but of sixteenth century Mexico. More than one reference is made by Halkuyt to the hides produced, and to the Spanish dairies of Mexico. While these are perhaps the earliest references to dairies in the New World, the places referred to were probably not dairies but cattle ranches. Due to the fact that dairies existed as agricultural enterprises in England at that time, the English narrator probably used the term he associated with herds of cattle, without thought as to the marketable product produced.

In 1572 the cattle business was flourishing in New Spain (Mexico) and it is written that one man had 20,000 head (27). Hides were the chief source of income, but a certain amount of tallow was shipped. Oxen were used at this date to haul goods, some of which was transported 700 miles.

Sir Walter Raleigh's expedition, under Sir Richard Grenville, to the Atlantic coast of the New World stopped at Hayti in 1585 where it was



entertained by the Spanish (27). "Which banquet being ended, the Spanish in recompence of our courtesie, caused a great herd of white buls and kyne to be brought to gether from the mountaines, and appointed for every Gentleman and Captaine that would ride, a horse ready saddled, and then singled out three of the best of them, to bee hunted by horsemen after their manner . . . the next day wee played the Marchants in bargaining with them by way of trucke and exchange of divers of their commodities, as horses, mares, kine, buls, goates, swine, sheepe, bull-hides, sugar, ginger, pearle, tabacco, and such like commodities of the Ilands."

The reference made by the English visitors regarding the cattle on Hayti gives proof that the early English colonists had knowledge of the West Indies as a source of cattle. The fact that this group of colonists purchased cattle and other classes of livestock on this island may justify the statement that the first livestock brought to the eastern coast of North America by the English was of Spanish origin. As this group of colonists, who landed on Roanoke Island, was mysteriously lost, the history of this importation of livestock must remain unwritten forever. In 1616 the English who settled on Summer Isles, or the Bermudas, purchased cattle for their plantation from the Spaniards in the West Indies (50).

In 1539 cattle were taken from Mexico into the present boundaries of the United States (38) and in 1541, 500 cows were taken across the Rio Grande by Coronado. As early as 1598 a large number of cattle, 4,000 in fact, was taken from New Spain, or Mexico, into what is now New Mexico by Don Juan de Oñate (20). The town of Santa Fe was established in 1609 and the cattle business was planted firmly in that section of the country. In 1769 cattle were taken from Mexico into what is now the state of California, by Serra and Portolá (31), and these were the nuclei for large herds that showed the influence of Spanish ancestry for many, many years.

It seems evident that by this time the cattle had become so numerous in the Spanish possessions in the New World they had no monetary value except as a source of hides and perhaps as a means of sport. This, of course, was exclusive of their value as beasts of burden. No references have been found that would indicate that the Spanish in the West Indies were users of dairy products or eaters of beef, although they seemed to esteem the flesh of swine, and kept large droves of them. From the Cabeza (33) description of the American bison and a comparison with the Spanish cattle of that day, the latter must have been rather large animals with long, heavy horns, and comparatively coarse flesh.

Cattle were taken into Florida by the Spaniards about the time the first permanent settlements were effected. In volume one of the *Colonial Records of Spanish Florida* (14) much evidence is presented concerning the first importations of cattle into Florida. These cattle were shipped from the Spanish islands of the West Indies, and apparently were kept on islands

along the coast of Florida. This was necessary because of the difficulties which arose with the Indians. Since these islands were not very productive, and since hostile natives prevented extensive crop cultivation, the raising of cattle was a difficult task. It was not until conditions permitted the production of cattle on the mainland that the cattle population reached significant numbers.

It is evident that among the inducements offered the colonists was the promise of livestock, for in 1576 dissatisfied colonists who wished to return to Spain claimed that they had not been given the cattle of all kinds that had been promised them, including "Twelve head with the bull." That the cattle were taken into Florida previously, however, there can be no doubt as the following evidence which grew out of an investigation made in Madrid by Licentiate Gamboa on matters concerning Florida will show.

"On being asked what kinds of cattle, large and small, the said Adelantado brought to the said province, and how he divided it up, to whom he gave it, and under what conditions: this witness said that he remembers that the first year he brought a certain number of cows, mares, hogs and goats, and he thinks there were sheep likewise; whereof the goats and sheep were eaten and consumed, and the Indians killed the hogs. He also knows and saw that the said cows and all of the mares were consumed and eaten by the soldiers and the other people without anything remaining; and this witness knows and saw that afterwards the said Adelantado again brought to the said country a quantity of cows and hogs, and he knows not how many; and they multiplied and there was stock raising in the land, especially in hogs." The date of this inquiry was February 5, 1573. This witness had gone to Florida six years before, and if cattle were taken at that time the approximate date would have been 1567.

A second witness who claimed to have gone from Spain to Florida in 1566 testified as follows: "At the beginning, which was about two years after this witness went there, as many as twenty horses and mares were brought, and twelve cows, forty hogs, thirty goats and a few sheep, which were all consumed and eaten because of the famine and want that occurred; and the Indians killed the hogs. Afterward the said Adelantado again stocked the land, with eighty cows, one hundred hogs, and another hundred in Santa Elena, and about twenty goats; which was all for the Adelantado, and none of it had been distributed; at least this witness does not know it."

Another witness who had gone to Florida about 1566 testified as follows: "As soon as he arrived in the land, he saw there cows, mares, goats, hogs and sheep; but that he does not know the number, not how they were apportioned, further than that, in the straits they were in, everything was eaten, and the Indians killed and ate the hogs."

Since many of the first colonists were placed in or about the fort on the island of Santa Elena they complained bitterly because of the lack of agri-

cultural possibilities, and the extreme difficulties in growing livestock. Even at St. Augustine the farming opportunities were limited, partly because of the insects and partly because of a scarcity of suitable feed.

When one witness was "Asked if there are in the country any cattle and vegetables wherewith the said people can sustain themselves, he said that in St. Augustine there were left fifteen or sixteen mares and ten or twelve cows; that the said cattle cannot sustain themselves because the mosquitoes eat them and the Indians kill them; . . ."

From these testimonials it is evident that the establishment of the cattle industry in Florida was accompanied by many difficulties.

The cattle brought into Florida were, without doubt, from the Spanish West Indies. With an abundance of cheap cattle so close at hand it would have been out of the question to ship them from more distant points. Cattle from the West Indies also found their way into South Carolina (25).

The influence of the cattle of Spanish origin on the characteristics of the cattle of Southern United States has never been appreciated fully by most students of livestock history in this country. When it is remembered, however, that all of the first cattle introduced into the southwest, and into Louisiana and Florida, were of Spanish origin and that large numbers of this type of cattle were introduced into practically every coastal colony, it must be admitted that the early influence of Spanish cattle was indeed great. One of the main reasons why native cattle in the South differ somewhat in conformation and utility from those in the North may be attributed to the original Spanish cattle in the southern part of the United States. The large numbers of cattle found in Florida in the early part of the eighteenth century as pointed out by Gray (25) lend further weight to the belief that Spanish cattle contributed an influence to our present day cattle that should not be minimized.

#### INTRODUCTION OF CATTLE BY THE FRENCH

The French, who made their first permanent settlements along the St. Lawrence, brought cattle to the American Continent as early as the middle of the sixteenth century. Cartier, when he sailed on his second voyage to the New World in 1541, had with him cattle, goats, hogs and other beasts. These were taken for breeding purposes in the new country (21). While this may have been the first introduction of cattle into the St. Lawrence watershed, yet it is apparently a fact that cattle were taken into that part of the world even before Cartier made his second voyage. When Sir Humphrey Gilbert reached St. Johns, Newfoundland, in August 1583 he learned from a native of Portugal that over forty years before some Portuguese had placed on Sable Island both neat cattle and swine for breeding purposes, and that these animals had increased greatly in numbers. The informing Portuguese claimed to have been present when this project was accom-

plished (21). Other historians (45, 54) have mentioned these cattle but their explanations are that these cattle had escaped from a wrecked Spanish or Portuguese ship. It is entirely possible that these animals may have been placed on Sable Island by the Portuguese since their presence there would constitute a distinct aid, from the standpoint of food, to Portuguese fishing vessels. Parkman (45), however, cites evidence that "in 1518 the Baron de Léry made an abortive attempt at settlement on Sable Island, where the cattle left by him remained and multiplied."

In regard to these same cattle on Sable Island Governor Winthrop of Plymouth (54) recorded on June 24, 1635, the following interesting account: "Mr. Graves in the **James**, and Mr. Hodges in the **Rebecka**, set sail for the Isle of Sable for sea-horse (which are there in great numbers) and wild cows. Mr. John Rose, being cast ashore there in the (*Mary and Jane*) two years since, and making a small pinnacle of the wreck of his ship, sailed thence to the French upon the main, being thirty leagues off, by whom he was detained prisoner, and forced to pilot them to the island, where they had great store of sea-horse teeth, and cattle, and store (of) black foxes; and they left seventeen men upon the island to inhabit it. . . . He saw about eight hundred cattle, small and great, and many foxes, whereof some were perfect black." On August 26 of the same year Winthrop wrote: "They returned from their voyage. They found upon the island sixteen Frenchmen, who had wintered there. . . . They had also killed many of the cattle, so as they found not above one hundred forty, and but two or three calves."

The French came to North America primarily to trade. Trapping for furs and trading with the Indians proved so remunerative that a permanent type of agriculture had little appeal. For several years the cattle that were kept supplied only some of the necessities of diet and had little commercial importance. Later, with the coming of the Jesuits, who had visions of a more self-sufficing New France, more attention was given to agriculture and stock-raising.

The first cattle brought into Canada by the French probably were of the type common to Brittany, for Cartier sailed from that region of France (45). Sanders (49) has written that "in 1620 a few cattle were landed at Quebec, and in 1665 Messers. Tracet and de Courcelle brought from France a small shipment described as black and brindle in color. These early selections were from Brittany, Normandy, and probably from the Island of Jersey, and their descendants to this day are not unlike the darker Jerseys in coloring." The present French-Canadian breed of cattle undoubtedly descended from the French types brought over by the early colonists.

The Jesuits, and the colonists who followed the paths of the religious trail blazers, brought cattle from the St. Lawrence Valley into the middle west. This did not occur until many years after the English and Spanish

had brought cattle to the Atlantic seaboard, but as foundation animals they exerted an influence on the early cattle of our present cornbelt area. Parkman (44) presents evidence which indicates that as early as 1649 the French had "fowls, swine, and even cattle" at Sainte Marie near the waters of Georgian Bay. Colonization occurred later at Detroit, and the area round about became populated, first by dependent Indians, followed by traders, and later by farmers and stock raisers. Carrier (13) points out that cattle were taken to Kaskaskia in 1712, and that Charlevoix, in 1721 found thriving settlements at these places with droves of "black cattle."

The first cattle that were taken into the lower Mississippi Valley by the French were, in most cases, of Spanish origin. In 1701 Iberville stopped at San Domingo and took on horses, cattle and swine for the new colony (Mobile) in Louisiana (29). On his first trip he had brought "a small number of bulls, cows, hogs, poultry, and turkey" (32). In 1703 four oxen were sent for in Havana (36). In 1704 there were 9 oxen, 14 cows and four bulls, and by 1708 there were 50 cows in milk, 40 calves, four bulls and 8 oxen (29). Cattle increased slowly in the French colony and many efforts were made, some which were successful, to obtain cattle from the Spanish islands near by. By 1724 there were 1100 cows, and 300 bulls in the colony (36), and by 1746 it was estimated there were 10,000 head of cattle in Louisiana (25). Here again the heavy influence of Spanish cattle in Colonial America must be acknowledged.

Hamilton in his book **Colonial Mobile** (29) has assembled much interesting information relative to the cattle population in the vicinity of that city. For example, he notes that in 1766 "there are from the highest to the lowest, on the east side of the Bay of Mobile, seventeen plantations, thirty-nine white men who can bear arms, thirty-two negroes of which twenty-nine are men grown, twenty-one women and children. In all, 124 souls and 2280 head of cattle." In another place he records that a man writing from Mobile in 1812 stated that he has "about 30 head of cattle and hundreds of hogs, the hogs wild." He also wrote that the cattle and hogs did well on his land with no expense. In 1814 when Andrew Jackson took over the territory in the vicinity of Mobile Bay, "There were a great many cattle east of the bay, the property of the Mobilians." This was the same area in which the census of 1766 revealed 2280 head of cattle. In the hundred years since Iberville first took cattle into that territory they had indeed made a very great increase. Similar development in cattle raising had taken place throughout the Gulf area before it became a part of the United States.

#### INTRODUCTION OF CATTLE BY THE DUTCH

While the English were colonizing Virginia and Massachusetts, and the Spanish were founding settlements in Florida and at Santa Fe, the Dutch had not been idle. In 1609 they established a trading post on the present site of Albany and by 1621 the settlement on Manhattan was started.

Four years elapsed before cattle were imported, but in **Narratives of New Netherlands** (52) it is stated that in November 1625 a ship arrived, and after unloading passengers "the cattle carried thither were removed upwards to a convenient place abounding with grass and pasture. Only two animals died on the passage. This gave great satisfaction to the freighter, who had managed the transportation so neatly."

The success of the first shipment of cattle led to further development along this line and by 1626 the colony had "increased to two hundred souls; and afterwards some ships, one with horses, the other with cows, and the third with hay; two months afterwards a fly-boat was equipped to carry sheep, hogs, wagons, ploughs and all other implements of husbandry. These cattle were, on their arrival, first landed on Nut Island, three miles up the river, where they remained a day or two." These cattle were taken later to Manhatas. "Being put out to pasture here, they thrive well, but afterwards full twenty in all died. . . . But they went in the middle of September to meadow grass, as good and as long as could be desired" (52). By this same authority it was pointed out that the West India Company of New Netherlands agreed to transport cattle free of charge for those patrons who were founding colonies.

The few details available relative to the importations of cattle into New Netherlands might indicate that relatively few were imported directly from Holland. On the other hand the importations of these animals may have been such a common occurrence that they did not elicit comment from the Dutch historians. Nevertheless, cattle played an important role in the agriculture of New Netherlands.

Under the early Dutch system of colonial agriculture "the Company furnished the farmer a house, farming implements and tools, four horses together with four cows, sheep and pigs in proportion, the usufruct and enjoyment of which the husbandman should have during six years, and on the expiration thereof, return the number of cattle received. The entire increase remained with the farmer. The farmer was bound to pay yearly 100 guilders and 80 pounds of butter rent for the cleared land and bowverie" (43). Here we see the strong influence of the homeland occupations guiding the colonists in the choice of a livelihood and a medium of exchange. In Virginia tobacco was used for money. In New England debts could be paid in terms of cattle. To the dairy-minded Dutch, however, the giving up of their cows was like surrendering ownership of the mine from which the gold is taken; and so they kept the cows and paid their rentals with butter.

About 1640 a war broke out with the Indians. Before this conflict ended the Dutch had lost a great many of their cattle at the hands of the Indians, and it was several years before they recovered from this loss. In 1650 a resolution by the States General forbade the exportation of cows

from the colony except by permission of the Council. In that same year, however, Cornelis van Tienhoven, secretary of the colony wrote (presumably in Holland) "Cattle, such as horses, cows, hogs need not be sent from this place, in consequence of the great expense, as they can be got at a reasonable price from the Dutch, and principally among the English, who have plenty of them" (43). This would indicate at least a partial recovery from the losses suffered during the Indian war, and it would also point definitely to the fact that the Dutch and English were on trading terms.

"Prior to the end of the Dutch regime, Long Island had been settled rather extensively with English farmers from New England and had become quite heavily stocked with cattle. Johnson gives 1640 as the date of the beginning of the English settlements on Long Island, but that was the date a church was organized. There were apparently individual settlers there a few years earlier" (13). This authority does not give the source from which the cattle came. They may have been from New England or Virginia, or they may have been purchased from the Dutch at New Amsterdam; for it is known that the Dutch and English carried on inter-colonial commerce (9). Denton (17), however, states "The Island is plentifully stored with all sorts of English cattle" and this would lead one to believe that most of the cattle were purchased from the English colonies.

The growing tendency of the English cattle to dominate, even within what the Dutch considered their own boundaries, is shown in a report of the conditions existing in 1649 within the Dutch limits as far East as Stamford. ". . . their cattle, including cows and horses, are computed at thirty thousand; their goats and hogs cannot be stated. . . ." Also, "Flushing, which is a handsome village and tolerably stocked with cattle; the fourth and last isheemstee, which is superior to all the rest, for it is very rich in cattle" (43). A reason for the trend towards English cattle is contained in this paragraph from a report, written in 1649, on conditions in New Netherland. "The domestic cattle are here in size and other respects about the same as in Netherland, but the English cows and swine thrive and feed best, yea, appear to be better suited to this country than those from Holland; they require also less trouble, expense and attention, for it is not necessary to look so much after the inferior stock, such as swine, in winter; but if done in some sort, whenever there is deep snow, t'will be so much the better. Milch cows, also are much less trouble than in Holland, for, most of the time, or when necessity demands, a little hay is only occasionally thrown to them" (43).

The influence of Dutch cattle in New Jersey is indicated by the following quotation. "When the English gained control in 1665 and undertook systematically to settle that part of America, East Jersey was already stocked with excellent horses and cattle, the original breeds coming from Holland and Sweden. It was early discovered that the improved animals

from the continent did not stand the adverse conditions of those early days as well as did the less improved English breeds. For that reason many animals were purchased in New England and brought to New Jersey. The crossing of the two strains gave a good general purpose breed" (13). Here is a direct inference that the cattle of Holland and Sweden were of higher quality and accustomed to better treatment than the English cattle. It is inferred also that of all the different nationalities that colonized on the North American continent the Dutch were superior in the field of animal husbandry. The English coming into New Jersey were willing to lose the higher production possibilities of the Dutch cattle rather than improve their own husbandry practices to the point where the Dutch cattle might have compensated them for their efforts. At such a price was a low level of production purchased as a foundation for many generations of American dairy cattle! That the good qualities of the Dutch and Swedish cattle were not all lost, however, is carried in the following description of the cattle belonging to John Bartram about 1750 as it was recorded by William Darlington (16). "His cows were then returning home, deep-bellied, short-legged, having udders ready to burst; seeking, with seeming toil, to be delivered from the great exuberance they contained."

It is unlikely that any cattle were imported directly from England as a basis for the early animal husbandry of New Jersey.

#### IMPORTATIONS BY THE SWEDES

Sweden's only attempt at colonization on the Atlantic coast was in 1638 (24). But it was not until 1640 that immigrants from Stockholm arrived with "cattle and implements of husbandry" (23). They settled in what is now the state of Delaware. Previous to this, in 1631, a Dutch ship, with colonists and cattle, had arrived on the Delaware river and a settlement was established, but it was destroyed by the Indians. Later cattle were imported from New Amsterdam and near-by territory and it is quite possible that cattle were purchased from the English in Virginia. At least this was suggested in the report of Governor Rising in 1654 (41). Although the Swedish influence as a colonial power lasted but a short time the influence of Swedish cattle was felt for a great many years. The people who came later into what is now Pennsylvania were glad to obtain good cattle from the Delaware Counties.

#### INTRODUCTION OF CATTLE BY THE ENGLISH

Although the quest for gold was the primary stimulus for English and Dutch explorations in the New World, they were willing to accept rich, productive land as a substitute for the precious metal. After the realization dawned that gold could not be dug out of every hill of the western hemisphere, groups in these respective countries turned their thoughts to the



serious consideration of colonization. Sweden, who wanted a home-made market for manufactured products, also decided to try her hand at colonization. So, at the beginning of the seventeenth century we find the zone of exploration, conquest and colonization shifting from the torrid to the temperate zone, and the white heat of the gold quest giving way to deliberate plans for the expansion of empires.

The first English colony was founded in 1607 at Jamestown in the present state of Virginia. By 1609 the colony was fairly well stocked with poultry, swine and sheep, and a few horses had been brought over. According to Captain John Smith (50) it was not until May 10, 1611, however, that cattle were first brought over from England. He also states that the next year, 1612, six ships bringing 100 "kyne" with other cattle arrived about the first day of August. While May 10, 1611, is the first date mentioned by Smith for the landing of cattle, Lord Delaware (47), who left the colony before the arrival of the ships on May 10 of that year stated in his *Relation* that "The cattell already there are much encreased, and thrive exceedingly with the pasture of that Country: The Kine all this last Winter, though the ground was covered most with snow, and the season sharpe, lived without other feeding than the grasse they found, with which they prospered well, and many of them readie to fall with Calve; Milke, being a great nourishment and refreshing to our people, serving also (in occasion) as well for Physicke as for Food, so that it is no way to be doubted, but when it shall please God that Sir Thomas Dale, and Sir Thomas Gates, shall arive in Virginia with their Extraordinary supply of one hundred Kine and two hundred swine. . . ."

There seems to be a discrepancy of one year's time between Smith and other chroniclers as to the exact date of the arrival of the first importation of domestic cattle into Virginia. It is possible that Delaware could have been mistaken were it not for the date of publication of his paper. Smith may also have been in error as to the date of the arrival of the six ships with 100 kine. He gives this date as August 1, 1612, while the records of Delaware (47), Hamor (30), and William Simmonds (40) indicate the date to have been 1611. Since these last three authorities wrote independently it is natural to conclude that they had the date listed correctly. If Delaware was correct in his statement that cattle were in Virginia before 1611 then we must conclude that the first importation was made in 1610.

Here it should be pointed out that there is some difficulty in distinguishing cattle from other domestic animals, when one is gleaning information from available literature. The terms "cattle," "cattell," or "cattel" were quite often used by early writers to include all kinds of domestic farm animals. Those of the bovine species were distinguished, quite often, from the others by the terms "neat," "horned," or "kyne."

The fact that the first colonists valued their animals highly is indicated

by the fact that they made careful preparation for their security and protection (50). The first cow stable was erected in Virginia in 1611 at the direction of Governor Dale (11).

Under the rule of Governor Dale the colony was brought to a fair degree of prosperity. In order to encourage the immigration of colonists certain very definite inducements were extended. Hamor (30) wrote regarding each colonist: "he shall be furnished with necessary tooles of all sorts, and for his better subsistence he shall have Poultry, and swine, and if he deserve it, a Goate or two, perhaps a cow given him." Although this livestock was loaned and not "given," this liberal policy on the part of Governor Dale was conducive to the rapid increase of livestock in Virginia, and the large number of domestic animals available was one of the chief inducements to families to come to America (1). That the preservation of the livestock was of greatest concern to the Plantation is shown in one of the provisions of the Martial Code enforced by Governor Dale. "No man shall dare to kill or destroy any bull, cow, calfe, mare, horse, colt, goate, swine, cocke, henne, chicken, dogge, turkie, or any tame cattle or Poultry of what condition soever. . ." (11).

Little is written about the special uses of cattle during the early days of the Virginia Plantations. It is known, however, that they were used as draft animals, for in 1614 Hamor (30) indulged in the hope that the following year three or four plows would be set to work, there being a sufficient number of steers at that time to draw them. Smith (50) in 1619 wrote of the need for men who could build and make carts and plows, and for skillful men who could train cattle to draw them. Mention is also made of the fact that in 1622 Captain Nuse shared with the starving members of the Colony his own portion of milk and rice, indicating the use of milk as a food for adults as well as for children.

In spite of the great interest in livestock, however, cattle seem to have multiplied slowly. By 1616 there was a total of only 144 head of cows, heifers, heifer calves, steers and bulls in Virginia (48) and in 1617 the number had decreased to 128. Argall, the Governor, sought to obtain an ample supply of winter feed for the livestock by prohibiting the use of hay in the preparation of tobacco for sale. When Argall fled the colony in March, 1619, however, all the *public* livestock had been killed except six goats; and when Sir George Yeardley took charge he had to make provision for supplying newcomers with cattle. In 1619 Sir Edwin Sandys proposed to the Virginia Company of London that 20 heifers be sent over, at a freight cost of ten pounds per heifer, to the colony for every 100 tenants. This would have amounted to 60 head in that year (1). On June 25, 1619, a shipment of corn (probably not *Zea mays*) and cattle was landed safely. By 1620 the total number of cattle in Virginia was estimated at 500 head. The twenty-second of November, 1621, a ship arrived from Ireland with

people, provisions, and cattle; and it is recorded in that year that 80 head of cattle were brought into Virginia (50).

There is much confusion as to the number of shipments of cattle leaving England and the number arriving in the colony. Losses of ships and of cattle were not unusual in those days and it is not possible to determine the exact number of cattle imported. From 1619 to 1622 there was a great deal of interest in the export cattle business to the colony. While it is noted that the Company required that cattle should be fine and spring from English breeds, yet it is a fact that many of the cattle came into Virginia from Ireland. Only female cattle were wanted at this time as there was a sufficient number of steers and bulls in the colony. A cow was valued in the colony at 15 pounds Sterling, and it cost 10 to 12 pounds Sterling to ship a heifer from England to Virginia. It is interesting to note that it cost only two pounds less to bring over a heifer than to bring over a man (11).

Whether by importations or by good husbandry, or a combination of the two, the number of cattle in Virginia had increased to 2000 by 1627. This estimate by Captain John Smith (50) included cows, bulls and oxen, and in 1629 this same authority recorded that in this year several people estimated the cattle population at about 5000 "kine, calves, oxen and bulls." As late as 1629, however, the Council of Virginia ordered that no healthy female cattle be killed unless they were non-breeders (26).

We may conclude that after about 1630 there were few importations of cattle into Virginia, except perhaps the occasional purchase of an outstanding breeding animal. This conclusion is based on the fact that by 1633 the youthful Plymouth colony in New England was buying cattle from the Virginians (18). Governor Winthrop of the Massachusetts Bay colony (54) recorded on August 3, 1636, that "Samuel Mavarick, who had been in Virginia near twelve months, now returned with two pinnaces, and brought some fourteen heifers, and about eighty goats." This is only one of many cases where definite records exist of exportations of cattle from Virginia. In 1631 it was ordered that each 20th calf, pig and kid should be given as a tithe to the religious minister (26). In 1649 there were 30,000 "head of Cattell, and an infinite number of Hogges," in Virginia (12) and in 1655 cattle were so plentiful that one cow was being offered as a bounty to the Indians for the bringing in of lots of eight each of wolf heads (26).

Smith (50) recorded that by 1629 there was a tendency to change from tobacco culture to a pastoral type of agriculture. "Jamestown is yet their chiefe seat, most of the wood destroyed, little corne there planted, but all converted into pasture and gardens. . . . Here most of their cattle doe feed, their Owners being . . . about their plantations. . . . Here in the winter they have hay for their cattell: but in other places they browze upon wood, and the great huskes of their corne, with some corne in them doth keepe them well."

Due to the difficulty of fencing, a large number of cattle ran at large and became wild. These cattle, however, could not be hunted without a license. Because of the range conditions existing, little provision was made for winter feeding and during the winter of 1673 it was estimated that 50,000 cattle perished because of the severity of the weather (11).

Although the first cattle taken into Virginia were under the strict supervision of the Company one should not be led to believe that the raising of cattle was entirely a public trust. As plantations increased in number, private herds came into being and increased both in number and size; and while it is estimated that the number of wild cattle, some of which were ear marked or branded, exceeded the number of tame cattle, yet there were several large herds kept in inclosures, and a few contained over 100 head each (11). From this time forward the increase in the number of cattle in Virginia continued until the beginning of the Revolutionary War.

The Plymouth Colony, although founded in 1620, did not import any "neat" cattle until four years later. We have Governor Bradford's statement (9) that in 1624 "Mr. Winslow came over, and brought a perty good supply, and the ship came on fishing, a thing fatall to this plantation. He brought 3 heifers and a bull, the first beginning of any cattle of that kind in the land. . . ." Faulkner (22) in commenting on this said "cattle were brought in as early as 1624 and formed the basis of rapidly increasing herds and successful dairying." Bradford (9) records further that in 1625 the factors of the colony from Plymouth, England, sent a shipment of cattle, cloth and other goods, in the custody of Mr. Allerton and Mr. Winslow, who were to sell them at their discretion. He further comments that "the cattle were the best goods, for the other being ventured ware, were neither at the best (some of them) nor at the best prices."

The idealistic system upon which the Plymouth Colony was founded did not function to the satisfaction of the colonists and in 1627 it was decided that goods and property should be divided among the members. "And first accordingly the few cattle which they had were divided, which arose to this proportion; a cow to 6, persons or shares, and 2 goats to the same, which were first equalized for age and goodness, and then lotted for; . . ." (9).

Captain John Smith (50) in commenting upon the founding of Salem, Massachusetts, in 1629 wrote: "In the yeare 1629, about March, six good ships are gone with 350 men, women and children: . . . Also 150 head of cattell, as horses, mares, and neat beasts; 41 goats. . . ." In discussing the islands at the mouth of the Charles River, Smith stated: "In the Isles you may keepe your hogs, horse, cattell, conies or poultry and secure for little or nothing." Thus did the colonists utilize this provision of nature to fence their livestock. He also recorded that in the summer of 1630 another ship arrived with twenty "cattell" and forty or fifty passengers. Bradford mentions a shipment of "kattle" brought over by Mr. Allerton and Mr.

Hatherby in 1630 and sold. A ship, the **White Angel** arrived at Sauco June 27, 1631, with "cows, goats, and hogs, and many provisions" (54). The record shows also that on July fourteenth of the same year the **Friendship**, of Barnstable, arrived at Plymouth and landed "eight heifers, a calf and five sheep." She had been at sea eleven weeks. On July 22 the **White Angel** that had arrived at Sauco nearly a month before landed 21 heifers at Plymouth.

Cattle importations hit a full stride in the 1630's, and the following are only a few of the recordings by Winthrop (54) relative to the bringing in of cattle. October 29, 1630; Mr. Goffe's ship "brought out twenty-eight heifers, but brought but seventeen alive."

June 12, 1632; The **James** arrived from London. "She brought sixty-one heifers and lost forty."

May, 1633; the **William and Jane** arrived . . . with thirty passengers and ten cows or more."

July 24, 1633; "A ship arrived from Weymouth, with about eighty passengers and twelve kine. . . ."

September 4, 1633; "The **Bird** arrived bringing some cattle."

October 10, 1633; The **James** arrived at Salem, "having been but eight weeks between Gravesend and Salem." She brought some sixty cattle.

It should not be supposed that the importation of cattle was without hazards. For example, Winthrop (54), reported the arrival of the **Mayflower** and the **Whale** at Charles Town harbor, July 1, 1630, with most of their cattle dead. The **Handmaid** arriving at Plymouth, October 29, 1630, lost 10 of 28 cows. The following are also reported by Winthrop:

October 29, 1630. Mr. Goffe's ship "brought out twenty-eight heifers, but brought but seventeen alive."

September 6, 1630. "The wolves did much hurt to calves and swine between Charles River and Mistick."

September 30, 1630. "The wolves killed six calves at Salem."

June 12, 1632. The **James** arrives from London. "She brought sixty-one heifers and lost forty."

On the other hand a few ships made the crossing without the loss of a single animal.

Winthrop (54) recorded at least 12 ships that brought cattle to the Massachusetts colonies during the years 1630-32. During 1633-34 at least 270 head of cattle were imported. He also reported the arrival of a ship from Texel, North Holland, in 1635 that brought 63 heifers.

It is interesting to note this early importation of Dutch cattle into the English colonies. It is reasonable to assume that the cattle introduced from Holland were similar to the ancestors of our present-day Holstein-Friesians. It is also interesting to speculate as to the possible relationship of the cattle of those early importations to our present breeds. One should bear in mind

that those early importations took place almost 250 years before the establishment of any of the registry associations for the maintenance of pure breeds of dairy cattle in the United States. We must go back to the time when cattle were designated as "black," "horned," "hornless," "short-horned," "middle horned," or "long horned" (3, 10, 15, 55). As these designations mean little to us to-day it is necessary for us to associate the cattle with the area from which they were shipped. Such a method is both reasonable and helpful.

It is logical to assume that cattle shipped from Plymouth and Barnstable came from the surrounding Devonshire area. In this area the Devon breed of cattle was developed. As there is no record of any mass movement of cattle to or from Devonshire during or following the colonization of North America it may be concluded that the first cattle brought to the New England colonies were of an inheritance similar to the Devon breed that was later developed and improved in Devonshire. The fact that Devon cattle have always been rather popular in the New England States adds further to the belief that the first cattle imported were of the Devon type.

The cattle that were shipped to the New England colonies from Virginia probably were of mixed origin. The first cattle brought to Virginia were of English origin. A little later Irish cattle of superior quality were brought in; and still later, when trade routes via the West Indies were established, Spanish cattle were imported by the Virginians. The result of importing from these various sources is presented well by Bruce (11) who, in writing of the Virginia cattle as they appeared in the seventeenth century, said, "from the variety of colors distinguishing the horned cattle entered in the appraisements, it would be inferred that there were no distinct breeds in the colony, the original ones having become by repeated crossings so confused in blood as to represent no separate types except in an extremely modified form." From this statement it is reasonable to conclude that the cattle shipped from Virginia into the Massachusetts colonies were of mixed inheritance and were, quite likely, inferior to the cattle imported from Devonshire and Holland.

Winthrop (54) recorded a shipment of cattle from Ipswich, and they were, no doubt, of the Essex and Suffolk type. He also noted a shipment from Gravesend and Southampton, and thus Hampshire and Kent made their contributions.

As the population of Massachusetts grew in numbers and the herds increased it became necessary for each man to enlarge his land holdings to take care of his livestock. "And no man thought he could live, except he had cattle and a great deal of ground to keep them; all striving to increase their stocks" (9). The Governor of Plymouth wrote in 1638, "It pleased God, in these times, so to bless the cuntry with such access and confluence of people into it, as it was thereby much enriched, and catle of all kinds stood

at a high rate for diverse years together. Kine were sold at 20 li and some at 25 li. a peece, yea, sometimes at 28 li. A cow-calfe usually at 10 li. A milch goate at 3 li, and some at 4 li. And femall kids at 30 s. and often at 40 s. a peece. By which means the anciente planters which had any stock begane to grow in their estats." Similar conditions prevailed in the Massachusetts Bay colony also, and in 1633, in writing of conditions Governor Winthrop (54) said: "They spent much in tobacco and strong waters, etc., which was a great wealth to the commonwealth, which, by reason of so many foreign commodities expended, could not have subsisted to this time, but that it was supplied by the cattle and corn, which was sold to new comers at very dear rates, viz., corn at six shillings the bushel, a cow at £20,—yea some at £24, some at £26,—a mare at £35, an ewe goat at 3 or £4; and yet many cattle were every year brought out of England, and some from Virginia." On November 17, 1636, he again wrote that "cattle were grown to high rates;—a good cow, £25 or £30; a pair of bulls or oxen, £40. Corn was now at 5 s the bushel, . . ."

People continued to flow into New England steadily and Winthrop recorded that in 1638 at least 3,000 people came over to the Massachusetts Bay Colony. The importation and raising of cattle brought prosperity to a height previously unknown in that part of the New World.

The law of supply and demand recognizes no territorial integrity, however, and we find that as a result of the great stimulus to stock raising an over-supply of cattle soon was in evidence. It may be that cows being priced from 25 to 28 pounds per head attracted shipments from across the ocean, as we know it did from Virginia. And so we read in Governor Bradford's History of the Plymouth Plantation (9) that in 1640 many began to fear a drop in the price of cattle. "And this was not a vaine feare; for they fell indeede . . . and that so suddenly, as a cove that but a month before was worth 20 li., and would so have passed in any payments, fell now to 5 li. and would yield no more; and a goate that wente at 3 li., or 50 s. would not yield but 8. or 10 s. at most. all men feared a fall of cattle, but it was thought it would be by degrees; and not from the highest pitch at once to the lowest, as it did, which was greatly to the damage of many, and the undoing of some." The same conditions prevailed throughout the Massachusetts Colonies (54).

This crash ended the commercial shipments of cattle from England to the colonies in New England. Their low value in the colonies was not equal to the cost of shipping cows from England to America. When prices recovered the New England demand was supplied from other colonies. By 1645 cows were selling at 30 pounds sterling and many were shipped from Virginia to the English colonies to the north (7).

Because of the comparatively late dates of the colonization of Maryland and Pennsylvania there is little question but that they obtained their cattle

from their neighbors to the east or to the south. Carrier (13) says, "The agricultural foundation laid by the early Dutch and Swedish settlers in New Jersey, New York and Delaware was of great value to Penn's followers. Here were excellent draft horses, oxen, Dairy cows and swine in numbers to supply all newcomers who possessed the necessary means to buy them." Cornelius Bon wrote in 1684, "I have a cow which gives plenty of milk" (35) indicating the availability of good cows. William Penn wrote in 1681 that newcomers "may as soon as they come buy cows more or less, as they want, or are able, which are to be had at easy rates" (41).

Rhode Island and Connecticut both probably obtained their first cattle from the neighboring English or Dutch colonists. There is no doubt of this in the case of Connecticut, and little doubt in the case of Rhode Island.

Although both the Dutch and the English had established forts or trading posts on the Connecticut River at an earlier date, the first real effort to found a permanent settlement was made in 1635. John Winthrop recorded (54) that on October 15, 1635, "about sixty men, women and little children, went by land towards Connecticut with their cows, horses and swine, and, after a tedious and difficult journey, arrived safely there." This is substantiated by Johnston (34) who writes that "In October of the same year (1635) a party of sixty persons, including women and children, largely from Newton, made the overland march and settled where Hartford now stands. Their journey was begun so late that the winter overtook them before they reached the river, and, as they brought their cattle with them, they found great difficulty in getting everything across the river by means of rafts." In Winthrop's history under the date of April 1, 1636, the following statement is found: "Those of Dorchester, who had removed their cattle to Connecticut before winter, lost the greater part of them this winter; yet some, which came late, and could not be put over the river, lived very well all the winter without any hay. The people also were put to great straits for want of provisions. They ate acorns, and malt, and grains. They lost near £2,000 worth of cattle." There is a further statement recorded on May 15, 1636, to the effect that "Mr. Hooker, pastor of the church of Newtown, and most of his congregation, went to Connecticut. His wife was carried in a horse litter; and they drove one hundred and sixty cattle, and fed of their milk by the way."

Less has been found relative to the introduction of cattle into Rhode Island. According to Carrier (13), among the early settlers were men of means who possessed livestock. In all probability some of these men went from the Massachusetts colonies and took cattle with them. Because of the strong feeling in Massachusetts against the Rhode Island group, however, there was little commerce between them. It is entirely possible that cattle were obtained from Virginia, or from the Dutch in the near-by colony of New Netherland. The fact that the dairy cattle of Rhode Island received



more than local notice at a very early date as being of very superior quality, would indicate a strong infusion of the Dutch cattle characteristics. In fact, before breeds of cattle were established in this country the "Rhode Island Cow" was well known as an excellent producer. The butter and cheese produced in that colony became known throughout the world and was an important item in the extra-territorial trade of the colony. William Douglass (19), in commenting upon the dairy industry of Rhode Island in the middle of the eighteenth century states: "The most considerable farms are in the Narraganset Country. Their highest Dairy of one Farm; *communibus annis* milks about 110 cows, cuts about 200 Load of Hay, makes about 13,000 wt. of Cheese, besides Butter; and sells off considerably in Calves and fatted Bullocks. A farmer from 73 milch Cows in five Months made about 10,000 wt. of Cheese; besides Cheese in a Season, one Cow yields one Firkin of Butter, 70 to 80 wt. In good Land they reckon after the rate of 2 Acres for a milch Cow." While the "Rhode Island Cow" does not exist as a breed at the present time she was the foundation of the commercial dairy cows, and contributed much to the profitableness of the dairy industry in the New England States.

According to Pirtle (46) the early "New Hampshire cattle were from the 'large yellow' Danish cattle." Allen (2) has pointed out that in 1631, 1632 and 1633 Captain John Mason imported cattle from Denmark for the Danish colonists in New Hampshire.

North Carolina was settled largely by people who left other English colonies in search of religious freedom, cheap land or security from persecution (4).

The first attempt at organized colonization was made in 1660 when a stock Company sent people from New England who settled near the mouth of what is now known as the Cape Fear River. These people had English Cattle on their plantations (42). The settlement was later abandoned and a portion of the cattle probably was left there. In 1664 an expedition was financed by people of Barbados, and a colony was established at Charles Town. Some cattle were raised here also but the enterprise failed. Whether these settlers took cattle into Carolina or appropriated those already there is a question that may be debated. They found cattle there, however, as is shown in this narrative of the first impression obtained upon arrival in 1664.

"We viewed the Cape-land, and judged it to be little worth, the Woods of it shrubby and low, the Land sandy and barren; in some places Grass and Rushes, and in other places nothing but clear sand; a place fitter to starve Cattel in our judgment, than to keep them alive; yet the Indians, as we understand, keep the English Cattle down there, and suffer them not to go off the said Cape, as we suppose, because the Countrey-Indians shall have no part with them, and as we think, are fallen out about them, who shall have the greatest share. They brought aboard our Ship very good and fat Beef

several times, which they could afford very reasonable; . . . .” And as a forecast of their own feelings at a later date they found a discouraging note which they answered thus—“Whereas there was a Writing left in a Post at the Point of Cape Fair River, by those New England-men that left Cattel with the Indians there, the Contents whereof tended . . . to the disparagement of the Land . . .” (39).

That the land did have livestock possibilities, however, is indicated by the description written in 1666 by Robert Horne. “The Marshes and Meadows are very large from 1500 to 3000 Acres, and upwards, are excellent food for Cattle, and will bear any Grain being prepared; some Cattle both great and small, which live well all the Winter, and keep their fat without Fodder; . . . .” But Cattle were not to be had easily as is indicated by this selection from a letter written by Governor Sayle and Council (39). Sept. 9th, 1670.

“Wee have received some cowes and hoggs from Virginia, but at an imoderate rate, considering the smalnesse of their growth. . . . If yor Honors had a small stoke in Bermuda from thence may be transported to this place a very good breed of large Cowes, Hoggs and Sheep at farr easier rates.” Definite progress was made, however, and Henry Brayne stated, in a letter to Lord Ashley, dated November 9, 1670, that he had “6 head of Cattle that my people have milk enough twice a day and that he had “there alsoe 7 hoggs,” three sheep, 6 geese, 8 turkeys and twelve chickens (51).

By 1622 the Carolinas were becoming “Cattle Country” and dairying was not unknown.

In Thomas Ashe’s description of Carolina in 1682 he wrote “The great encrease of their Cattel is rather to be admired than believed; not more than six or seven years past the Country was almost destitute of Cows, Hogs and Sheep, now they have many thousand Head.” And he also wrote that “The Cows the Year round brouzing on the sweet Leaves growing on the Trees and Bushes, or on the Wholesome Herbage growing underneath; They usually call them home in the Evening for their Milk, and to keep them from running wild” (39).

In the same publication, in Wilson’s account of Carolina in 1682 we find that “Neat Cattle thrive and increase here exceedingly, there being particular Planters that have already seven or eight hundred head, and will in a few years in all probability, have as many thousands, unless they sell some part; . . . .”

Because of the low cost of production due to year around pasture it was the expression of Wilson that—“many judicious Persons think that Carolina will be able to supply those Northern Collonys, with salted Beef for their Shipping, cheaper than they themselves with what is bred amongst them; for, considering that all the Woods in Carolina afford good Pasturage, and the small Rent that is paid to the Lords Proprietors for Land, an Ox is raised at almost as little expence in Carolina as a Hen is in England.”

Archadle (39) was also enthusiastic about the livestock possibilities of the Carolinas for he wrote that "so advantageously is the Country scituated, that there is little or no need of Providing Fodder for Cattle in the Winter; so that a Cow is grassed near as cheap as a Sheep here in England. . . ."

The place of Cattle and livestock and dairy products in the economy of the Carolinas is set forth in the letters of Thomas Newe (39) written in 1682.

"Severall in the Country have great stocks of Cattle and they sell so well to new comers that they care not for killing, which is the reason provision is so dear in the Town, whilst they in the Country are furnished with Venison, fish, and fowle, by the Indians for trifles, and they that understand it make as good butter and cheese as most in England." And in speaking of the circumstances of the first settlers who came to the Carolinas, Newe continued—"few of them having wherewithall to purchase a Cow, the first stock whereof they were furnished with, from Bermudas and New England, from the latter of which they had their horses which are not so good as those in England, but by reason of their scarcity much dearer, an ordinary Colt at 3 years old being valued at 15 or 16 *lis.* as they are scarce, so there is but little use of them yet, all Plantations being seated on the Rivers, they can go to and fro by Canoo, or Boat as well and as soon as they can ride, the horses here like the Indians and many of the English do travail without shoes. Now each family hath got a stock of Hogs and Cows, which when once a little more encreased, they may send of to the Islands cheaper than any other place can, by reason of its propinquity, which trade alone will make it far more considerable than either Virginia, Maryland, Pensilvania, and those other places to the North of us."

By 1728 cattle were apparently plentiful near the Virginia-Carolina line for they were found roaming at large and subsisting on natural feeds throughout the winter season (4).

South Carolina was colonized for the primary purpose of producing tropical and semi-tropical plants of economic value. The ideal grazing conditions and the extremely light winters obtaining in that area, however, caused cattle raising to become the principal occupation at an early date. Cattle were purchased from Barbados, the Bermudas, Virginia and New York. The cattle from Virginia were small and high in price. Those from New York were large and very heavy milkers, and the colonists preferred them to the cattle from Virginia or Barbados. As an indication of the development of the cattle industry Governor Nicholson of Maryland in 1695 spoke of the "vast flocks of cattle" in the Carolinas, and Nairne wrote that South Carolina had more "black cattle" than any other English colony (25).

The continuous reference to black cattle by many of Gray's authorities (25) has led him to the conclusion that most of the southern cattle of colonial and post-Revolutionary days were black and descendants of the early Spanish cattle. If this is true, the foundation for our southern cattle probably

had few of the qualities so greatly desired in a dairy animal. This may explain in part the low production of the average cow of the southeastern part of the country.

It is entirely possible, however, that Gray was mistaken when he concluded, because of the numerous references to "black cattle," that most of the cattle of colonial America were black in color. A more plausible explanation of the term "black cattle" is contained in the following statement by Cadwallader John Bates (5) who, because of his familiarity with the history of livestock improvement, may be accepted as a good authority. "So prevalent was the black colour in the North of England and the South of Scotland that bulls, cows, and oxen were given the generic name of 'black cattle.' Originally the Scottish thieves appear to have called the 'black cattle' they were driving off, their 'blackmail' or 'black-rent'; the terms being afterwards applied to the money paid them for foregoing these exactions in kine." Because the designation "black cattle" was in such common usage in England during the seventeenth century it is quite possible that the term was not truly descriptive of the color of the American cattle population of that day. That the greater portion of the cattle in the southeastern part of the Colonial North America was of Spanish origin can scarcely be questioned, but to say that they were black is perhaps imposing upon the available historical evidence.

According to Carrier (13) the foundation cattle of Georgia were purchased in South Carolina. It is quite probable also that cattle from Florida found their way into Georgia.

#### SUMMARY

The data contained in the literature reviewed, points to the fact that cattle were imported directly to Virginia, Massachusetts, New York, New Hampshire, Delaware, and possibly southern New Jersey, from the colonizing European countries. Many cattle, however, were brought into the southwest, the Gulf area, Florida and the southeast from the Spanish possessions in the West Indies and from Mexico. It also appears that many cattle containing at least some Spanish inheritance were shipped into Virginia, Delaware, New Jersey, and Massachusetts.

The initial mass importations of cattle from Europe into the North American colonies ceased about 1640. From that date to the American Revolution the cattle needs of the colonies were taken care of through inter-colonial trade, or through trade with the Spanish colonies in the Western Hemisphere. A few cattle from the French Colonies in the St. Lawrence River Valley found their way into the "Old Northwest."

The cattle improvement era did not start in England until many years after the initial period of importations into America had closed, and in America there was no basic work in cattle improvement during that period.

American breeders waited for the English and European stockmen to supply the superior breeding stock which was so necessary in grading up the common cattle that by 1800 had increased to several million head.

From 1640 to 1800 there was only an occasional animal imported and the only real possibility for general improvement of the milch cows lay in selection from within the existing cattle population.

From 1800 to 1860 there were few attempts to protect the "purity" of the improved cattle which were being imported from time to time. The efforts made in the past 80 years to improve cattle in general, and particularly dairy cattle, have not been sufficient to eliminate all of the influence of the cattle that were bred in America for the first 250 years. To be convinced of this fact one needs only to travel through the southeastern part of the United States—the oldest cattle country in our nation.

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# THE ANATOMY AND PHYSIOLOGY OF THE TEAT SPHINCTER\*

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Three workers (1, 2, 3) have reported that a slight negative pressure develops at the external opening of the teat when the pressure of milking is released. This negative pressure would tend to draw bacteria into the teat canal if they were present at the teat opening. If this is true, when cows are machine milked the constant vacuum within the teat cup should prevent any danger of infection during the milking process. This seems quite important in view of the great amount of trouble which dairymen are having with mastitis at the present time.

Since the upper end of the teat canal opens directly into the gland cistern it is difficult to see how there could be any negative intra-teat pressure unless the teat walls have certain elastic properties. As the teat walls are quite vascular and as experimental evidence has indicated that the "letting down" of milk is associated with increased tonus of the smooth muscle of the udder, it is conceivable that the teat walls might develop a certain tone (a) by contraction of the smooth muscle present or (b) by "erection," due to increased blood pressure. Because the teat does not tend to diminish in diameter when the "letting down" reflex is greatest, it seems logical to assume that any rigidity which the teat wall possesses at milking time is not due to a contraction of the circular or longitudinal muscles fibers in the walls of the teat.

The second theory to be considered is that the teat walls become turgid due to filling of the vascular bed of the teat at milking time. To test this theory a cow which had freshened recently was milked just enough to cause her to let down her milk. A small amount of barium paste was then quickly injected into two teats at a time and x-ray pictures taken before the increased tonus had lessened appreciably. Figure 1 A is a typical picture of the teat at this time. A milking tube was then inserted into each teat to relieve the pressure of the milk on the inner walls and another series of pictures taken. A typical picture of this series is shown in figure 1 C. About half an hour later, after the cow had been milked and all reflex activity had disappeared, barium was again injected into the teat canal and a third series of pictures taken (fig. 1 B). From these pictures it is apparent that the teat walls do not become engorged with blood when cows "let down" their milk. In other words, it appears that erection of the teat walls isn't necessarily associated with "letting down" of the milk in the udder.

Another type of experiment was then performed to study the vascular

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supply to the udder. The cow used was in milk and had a fistula of the rumen. By inserting the hands into the rumen through the fistula and pressing the fingers against the dorsal wall, it is possible to close the left external iliac artery. This is the chief source of arterial blood to the udder. Although the closing of this artery also shuts off the blood supply to the left limb and adjacent parts, no reason was seen why this should interfere with the experiment. Just prior to the beginning of milking, an assistant closed the left external iliac artery as just described. The milker then attempted

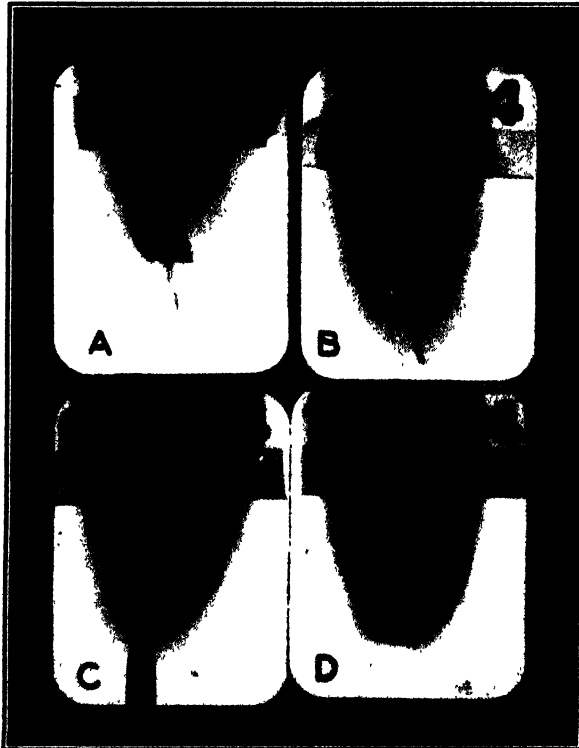


FIG. 1. A. X-ray picture of teat wall and cistern during maximum tonus of mammary gland. B. X-ray picture of teat wall and cistern one-half hour after milking. C. Same as A except that milking tube has been inserted into teat to relieve milk pressure. D. X-ray picture showing small amount of barium in the end of lactiferous duct.

to empty both sides of the udder at an equally rapid rate. At the same time he tried to discern any difference in tonus of the two halves of the udder. When milking was about half completed still more careful observations were made. The left external iliac was then released. Repeated trials failed to show any difference in the turgidity of the teat wall with the artery occluded. These observations along with the results of the x-ray pictures convince the authors that erection of the teat is not an important physiological process in

milking. If erection does not occur it is difficult to understand how a negative pressure could develop at the teat orifice on release of the positive pressure of milking.

In order to study the theory of negative intra-teat pressure the experiment by Davis (1) was repeated with the following modifications. Instead of inserting the teat opening into a dye solution as milking pressure was released, the teat was inserted into a suspension of barium. An x-ray picture was then taken of the end of the teat. No barium could be detected in the lactiferous duct. This procedure was repeated with twenty cows. A commercial preparation of lipiodol was also tried on two cows, with negative results. Although these trials would tend to indicate that the negative pressure is negligible it is possible that the barium was strained out by the smallness of the opening or the barium concentration was too dilute to be registered on the film.

On numerous occasions it had been noted that the teat sphincter tended to evert slightly while milk was being forced from the teat. A barium paste was therefore rubbed over the end of several teats when the greatest pressure possible was exerted without causing the milk to escape. X-ray pictures indicated that on release of this pressure a certain amount of barium had been drawn into the lower portion of the lactiferous duct. A picture, typical of these results, is shown in figure 1 D.

Various workers have expressed the belief that the healthy cell protects itself from bacterial invasion by secreting a substance having bacteriostatic properties. If the teat sphincter closes normally, practically all of the milk is forced out. Even though a slight contamination of the lower end of the canal does occur, as was shown with barium in figure 1 D, it is evident that the chances are slight for bacteria to migrate the remaining distance into the teat cistern and thence gain entrance into the udder. If the teat sphincter is patent or the lining of the duct becomes infected and rough the chances would seem much greater for bacteria to gain entrance into the udder.

Although anyone can detect the difference between a hard milker and an easy milker it is difficult to give an accurate numerical value to differences in tone of the teat sphincter, especially when these differences are slight. A standard Tycos sphygmomanometer was tried. In place of the usual rubber bladder and sleeve band a small rubber bladder and sleeve was made to fit about the teat. The teat was then closed at the base with a pair of rubber covered clamps just sufficient to prevent the milk in the teat from returning to the gland cistern. Readings were made when pressure on the bladder by the hand caused a very small stream of milk to be forced from the teat.

This device gave only fair results even after considerable experience in its use. The personal element of gauging the size of the stream could not be ruled out. But even more serious than that was the standardization of the physiological conditions. Although the clamp was applied at the base

of the teat with just enough force to prevent the milk in the teat from escaping when readings were being made this pressure exerted an unpredictable effect on the teat sphincter. Even with fairly hard milkers the tightening of the clamp might cause the teat to begin leaking milk even when the teat was only half full. These results could not be consistently duplicated nor can a satisfactory explanation be given for these inconsistencies.

Still another difficulty was found in making accurate determinations of the milking pressure. Not infrequently the mercury might rise 100 mm. in the manometer before milk would begin to flow, while after the flow had begun a pressure equivalent to 50 mm. of mercury would maintain the same degree of flow. From x-ray pictures just before and during the time that pressure was applied to the teat it is evident that the canal through the teat



FIG. 2. Teat showing healthy condition of tissue about the teat orifice.

sphincter is reduced from about 12 mm. to 8 mm. in length. At the same time the teat cistern tends to balloon out as the milk in the teat cistern is put under pressure until the folds in the mucosa disappear. It is quite possible that this tension also flattens out Furstenberg's rosette until it no longer interferes with the escape of the milk. When pressure is applied slowly in making readings with the sphygmomanometer it is possible that folds of the rosette may temporarily block the internal orifice of the teat canal.

In the light of the few cases observed and due to the variability in readings of milking pressures it is not possible to tell whether easy milking cows are more susceptible to infection with mastitis than hard milking cows. Certainly, there is no definite trend.

On numerous occasions the milkers of the Experiment Station herd have mentioned the frequency with which soreness at the end of the teat is associated sooner or later with the development of mastitis in that quarter.

In most of the cases referred to there seems to be a tendency for the teat sphincter to remain slightly everted and become eroded. A severe case of erosion is shown in figure 3. This should be compared with the healthy teat shown in figure 2. All gradations of this condition were noted in the herd.

Since the condition was much more prevalent among the machine milked cows it seemed important to find out whether the withdrawal of milk from the teat by suction, as in machine milking, was more severe on the teat than forcing the milk past the sphincter, as in hand milking. The company which manufactures the milking machine used very kindly supplied us with a transparent teat cup. It was quite evident from watching this cup in operation that on the release of the vacuum outside the rubber teat cup liner the teat is compressed over its entire length. This compression of the teat forces



FIG. 3. Teat showing eroded condition of tissue about teat orifice.

the teat cup down and probably gives the teat a better chance to fill on the application again of vacuum to the outside of the teat cup liner. During the entire milking process there is no release of the vacuum on the end of the teat. In order to see what effect this vacuum might have on the teat, the inner rubber liner was cut off just high enough to allow the teat to show at the end of the liner when the vacuum was applied. Cutting the liner made it necessary to shut off the pulsator line but normal milking could be maintained by pulling down on the teat cup every time the adjoining cup was forced down by the pulsator. As mentioned before, this motion apparently prevents the teat cup from squeezing the teat canal shut at the base of the teat.

In spite of the so-called pulsator device on most milking machines, the withdrawal of the milk is a continuous process once the machine has been attached to the cow. Although a properly operated machine should be able to

remove the milk from the udder more rapidly than can be done by hand milking, due to the continuous withdrawal of milk from all quarters, the continued application of a high negative pressure to the end of the teat may offset this advantage. A large number of herds should be studied over a period of years to adequately determine what effect long continued negative pressures have on the teat, especially the teat sphincter. In this experiment when vacuum was applied for two minutes the end of the teat became red and appeared badly congested. The congestion disappeared on breaking the vacuum in the teat cup. Although the cutting of the teat cup liner prevented the machine from functioning normally, it seems quite evident that the constant suction on the end of the teat is in part responsible for the eroded condition shown in figure 3. In order to verify this theory, a herd of over two hundred milking cows which have always been milked by hand was examined for this condition. This herd contains several of the heaviest milking cows in the United States, some of those examined having produced over one thousand pounds of fat in a year on official test. Although three cows in this herd had one or more quarters with slightly eroded sphincters, the difference was sufficiently great between this herd and our own as to offer substantial evidence of the danger of carelessly operated milking machines. A large proportion of the many cows in the college dairy herd show perfectly healthy teats after having been milked for years by machine but it is quite evident that certain cows are more sensitive to this type of milking than others. In most cases the teat orifice of hand milked cows appears as a slight depression at the end of the teat. With machine milked cows the opening of the teat is more likely to appear raised even though erosion of the sphincter lining has not occurred. Certainly extreme care should be exercised with cows showing any tendency toward irritation after machine milking, especially in not allowing the machine to remain on too long.

#### SUMMARY

No evidence was obtained to indicate that a negative pressure develops at the external orifice of the teat when the pressure of milking is released.

With machine milked cows there is a greater danger of injury to the teat sphincter than with hand milked cows. A teat whose sphincter becomes eroded at the external orifice seems to offer a greater opportunity for infection of that quarter than one which shows no erosion.

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# ACCURACY OF LIVE WEIGHTS OF DAIRY COWS ON PASTURE<sup>1</sup>

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## INTRODUCTION

The importance of accurate live weight records in investigations with dairy cattle can hardly be over-emphasized. This is particularly true in pasture experiments where live weights form the basis of calculating the nutrients required for maintenance and live weight changes of animals during grazing periods. The influence of variation in live weight of grazing animals upon the yield of experimental pastures has been the subject of considerable discussion among investigators working with pastures (5). Some have expressed the opinion that variations in live weight may be so large that a satisfactory measurement of the yield as expressed in terms of the grazing animal cannot be attained. Little work has been done, however, to determine the significance of variations in live weights of cows in pasture experiments. An almost universal recommendation has been to base the initial and final weight on the average of three successive days weighings.

Lush and Associates (4) reported that the probable error of a one-day weight was 4 to 8 pounds and that by weighing two additional days 42 per cent of this error was eliminated. These results were obtained from feeding experiments with steers, cows and heifers, largely in dry lot, conducted at different experiment stations in this country. Brown (1) and Brown and Slate (2), using steers in pasture experiments, found that two-day weights reduced the probable error over a one-day weight by 29 per cent. Thirty-three per cent of the two-day weights of steers varied less than 6 pounds and only 10 per cent varied more than 20 pounds. The following is an analysis of the variation in the live weights of lactating dairy cows used in grazing experiments.

## EXPERIMENTAL

The data used in these analyses were taken from a grazing experiment reported in detail in another place (3). Holstein-Friesian cows grazed in five-acre pasture at periodic intervals during each of five seasons. Four or five grazings were obtained each year. Grazing was at the average rate of about 2.5 cows per acre during the time the cows were on the pasture. The

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cows were required to graze the pasture uniformly after which they were removed to other pasture of comparable character until the experimental plot had made new growth. Grazing was begun when the grass had grown to a height of 6 to 10 inches, depending upon the season.

The cows were maintained on pasture day and night except for the time they were removed for milking. They were milked twice daily and were out of the pasture about 3 hours morning and evening. They were fed a small amount of supplementary feed at this time (the nutrients consumed as supplement constituted an average of only 4 per cent of the total nutrient requirements of the animals).

Initial and final live weights of each cow were taken, to the nearest pound, on three successive days at the beginning and end of each grazing period. The initial weights were the averages of weights taken the two days preceding and the first day of the grazing period. The final weights were the averages of weights taken on the last two days of the grazing period and the day following. The cows were always weighed in the afternoon following milking.

The experimental errors, the standard deviation of the daily trend of the total weight of each group of cows and the standard errors of the mean

TABLE 1

*Live weights of cows used in grazing experiment taken on three consecutive days at the beginning of the first grazing period in 1936\**

Cow No.	Weights of cow on			Total
	May 4	May 5	May 6	
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
124	1283	1267	1284	3834
129	1355	1353	1358	4066
133	1170	1165	1188	3523
134	1276	1285	1294	3855
135	1231	1235	1255	3721
138	1210	1221	1201	3632
145	1121	1139	1125	3385
147	1219	1221	1218	3658
148	1286	1288	1299	3873
150	1104	1078	1124	3306
153	1097	1108	1127	3332
157	1082	1082	1076	3240
160	1162	1177	1171	3510
161	1155	1170	1135	3460
168	1043	1008	1025	3076
179	982	986	980	2948
181	1015	1053	1047	3115
187	1130	1142	1135	3407
188	1127	1137	1121	3385
189	1127	1126	1097	3350
Total	23175	23241	23260	69676

\* This table illustrates the basic data used for analysis of variance in the initial and final live weights of the cows used in each grazing period.

initial and final live weights of the cows for each grazing period were determined by analyses of variance (6).

### RESULTS

Table 1 illustrates the original data from which the analysis of variance in live weights was made. It gives the weights of the individual animals in the group for three successive days for the beginning of the first grazing season in 1936. Table 2 shows the analyses of variance in these weights and is fairly representative of all such tables. Similar analyses were made of

TABLE 2

*Summary of analyses of variance of initial live weight of cows used in the first grazing period, 1936\**

Variation due to	Degrees of freedom	Sum of squares	Mean square	Standard deviation
All causes	59	542337.7	9192.16	
Daily changes†	2	199.0	99.50	
Difference between animals	19	536346.4	28228.76	
Remainder, experimental error	38	5792.3	152.43	12.34

\* Similar analyses were made for initial and final live weights of cows used in each grazing period.

† Represents day to day changes in the weight of the entire group of animals.

the initial and final weights for each grazing period, 46 in all. The experimental errors, *i.e.*, the square root of the mean square error, are given in table 3. There was considerable variation in the experimental errors, which did not appear to show any consistent differences with advancement of the pasture season, with different years, or with initial versus final weights. The weighted average experimental error was 14.0 pounds and the range was from 7.0 pounds to 28.3 pounds.

The standard deviation of the day to day changes in the weight of the whole group of cows is depicted in table 4. This was obtained by dividing the square root of the mean square of the daily changes (see table 2) by the square root of the number of animals in the group. The average day to day change in the group weight for all 46 weigh periods was found to be 7.7 pounds with a range from 0.5 pounds to 20.8 pounds. The deviations of the initial weights and the final weights were of about the same magnitude. This variation in day to day changes of the weight of the whole groups of cows is not greatly significant as indicated by the fact that in only nine instances out of the total of 46 did it exceed the size of the experimental error (standard deviation of remainder). The average was only half that of the experimental error.

The standard error of the average initial and final live weights and the average gain in live weight of the cows for each grazing period is given in table 5. The standard error was obtained by dividing the experimental



TABLE 3

*Experimental errors of initial and final weights of cows for the various grazing periods*

Grazing period	Degrees of freedom of the remainder	Experimental error of initial live weights	Experimental error of final live weights
		<i>lbs.</i>	<i>lbs.</i>
Pasture season of 1936			
1	38	12.3	14.2
2	28	13.6	17.2
3	28	10.3	11.8
4	28	13.0	13.5
5	28	8.4	7.0
Pasture season of 1937			
1	32	11.7	9.2
2	28	14.7	10.5
3	28	13.9	17.2
4	26	15.6	13.9
5	22	28.3	11.0
Pasture season of 1938			
1	20	11.2	11.1
2	18	11.5	16.0
3	22	16.4	13.2
4	22	14.3	19.1
Pasture season of 1939			
1	22	7.5	10.9
2	24	12.9	14.5
3	22	16.6	13.3
4	18	13.7	10.0
Pasture season of 1940			
1	20	10.9	11.8
2	18	10.4	13.5
3	18	14.8	19.2
4	22	12.6	13.4
5	22	19.3	15.0

error by the square root of the product of the number of cows in the group times the number of days weighed. The average standard error of the initial live weights was 2.2 pounds with a range of from 1.2 pounds to 2.9 pounds, while that of the final live weights was 2.2 pounds with a range of 1.0 pounds to 3.6 pounds. The average standard error of the gains was 3.1 pounds with a range of from 1.6 pounds to 5.0 pounds.

The results of these analyses compare favorably with those obtained by Lush and Associates (4), who found an average experimental error in three successive days live weights of cattle of 10.0 pounds (range 4.0 to 17.0 pounds) and a probable error<sup>4</sup> of 1.0 to 2.0 pounds and a probable error

<sup>4</sup> The probable error is 0.6745 times the standard error.

TABLE 4

*Standard deviation in day to day changes in live weights of groups of cows*

Grazing period	Number of cows	Standard deviation of daily changes	
		Initial weights	Final weights
Pasture season of 1936			
1	20	2.2	11.9
2	15	2.8	20.8
3	15	1.9	8.8
4	15	6.6	4.7
5	15	3.3	3.2
Pasture season of 1937			
1	17	5.3	2.4
2	15	10.3	13.7
3	15	4.7	3.0
4	14	3.7	5.9
5	12	6.2	17.6
Pasture season of 1938			
1	11	11.0	16.3
2	10	0.5	6.1
3	12	12.5	12.7
4	12	3.8	8.0
Pasture season of 1939			
1	12	4.8	2.0
2	13	4.3	7.9
3	12	4.2	10.4
4	10	6.3	3.5
Pasture season of 1940			
1	11	10.6	7.2
2	10	11.6	4.1
3	10	2.6	8.4
4	12	16.0	7.9
5	12	20.7	19.9

for gains in weight of 1.0 to 3.0 pounds. Under the conditions existing in the present experiment the variations in live weight of milking cows grazing on pasture were approximately the same as found by them for cattle maintained under feed lot conditions. The small standard errors obtained indicate the reliability of the live weight data used in calculating the total digestible nutrient yield of pasture in this experiment.

#### CONCLUSIONS

The average experimental error of live weights of cattle weighed on three successive days as determined by analyses of variance was 14.0 pounds. The range of 46 groups of weights was from 7.0 pounds to 28.3 pounds. The standard deviation of day to day changes in the weight of the groups of cows

TABLE 5

*Average initial and final live weights and gain in weights with the corresponding standard errors of cows grazing on pasture*

Grazing period	Number of cows	Average initial weight	Average final weight	Average gain in weight
		lbs.	lbs.	lbs.
Pasture season of 1936				
1	20	1161 ± 1.6	1207 ± 1.8	46 ± 2.4
2	15	1205 ± 2.1	1246 ± 2.5	41 ± 3.3
3	15	1239 ± 1.5	1274 ± 3.0	35 ± 3.3
4	15	1277 ± 1.9	1313 ± 2.1	36 ± 2.8
5	15	1301 ± 1.2	1339 ± 1.0	38 ± 1.6
Pasture season of 1937				
1	17	1165 ± 1.6	1224 ± 1.3	59 ± 2.1
2	15	1261 ± 2.2	1281 ± 1.6	20 ± 2.8
3	15	1306 ± 2.1	1332 ± 2.5	26 ± 3.3
4	14	1241 ± 2.4	1280 ± 2.1	39 ± 3.1
5	12	1260 ± 4.8	1298 ± 1.8	38 ± 5.0
Pasture season of 1938				
1	11	1217 ± 1.9	1298 ± 1.9	81 ± 2.8
2	10	1311 ± 2.1	1369 ± 3.0	58 ± 3.6
3	12	1279 ± 2.8	1352 ± 2.2	55 ± 3.6
4	12	1293 ± 2.4	1321 ± 3.3	28 ± 4.0
Pasture season of 1939				
1	12	1069 ± 1.2	1167 ± 1.8	98 ± 2.1
2	13	1228 ± 2.1	1275 ± 2.4	47 ± 3.1
3	12	1255 ± 2.8	1304 ± 2.2	49 ± 3.4
4	10	1207 ± 2.4	1248 ± 1.8	41 ± 3.0
Pasture season of 1940				
1	11	1091 ± 1.9	1177 ± 2.1	86 ± 2.8
2	10	1234 ± 1.9	1256 ± 2.5	22 ± 3.1
3	10	1219 ± 2.8	1230 ± 3.6	11 ± 4.5
4	12	1142 ± 2.1	1185 ± 2.2	43 ± 3.0
5	12	1145 ± 3.3	1182 ± 2.5	37 ± 4.2
Range in standard error 1.2 to 4.8			1.0 to 3.6	1.6 to 5.0

averaged 7.7 pounds. The standard error in the weights of 1200-pound cows was on the average only 2.2 pounds.

It is concluded that the method of weighing used in this experiment gave an accurate measure of the live weights of the cows from which the nutrient yield of the pasture was calculated.

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# REPORT OF A STUDY ON THE TOXICITY OF SEVERAL FOOD PRESERVING AGENTS<sup>1</sup>

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The deterioration of some foods caused by the activity of microorganisms may be prevented and the keeping quality of the foods greatly improved by the addition of certain chemical compounds. It is obvious that preservatives which produce a marked toxic effect should not be used in food intended for human consumption. On the other hand, even when a marked toxicity is not shown, the consumption of food containing unusual quantities of preservatives may be dangerous and harmful because of the hidden cumulative effect.

Definite proof of toxicity lies in knowing how the chemical agent is handled in the body. By tracing the chemical reactions of the preserving agent in the animal body and determining the method by which it is eliminated from the body, an accurate statement concerning its toxicity can be made.

This experiment was designed to yield information that may contribute to the knowledge of the toxic properties of certain chemical substances used in human food, and a bactericidal material used for sterilizing utensils. The amount of gain made by growing white rats was used as an indication of the toxic effects. Individual feeding trials were conducted with diets to which were added calcium propionate, sodium propionate, sodium benzoate, zephiran, and sodium benzoate plus glycine. Propionates are used in the bread industry and to some extent in packaged cheese in order to control mold. Zephiran is a new germicide which may be used for disinfecting dairy utensils. Sodium benzoate has been used for some years as a preservative for various food products.

Griffith (1) has shown that when 1.5 per cent or more sodium benzoate was in the diet, the rate of growth in young rats was decreased. The excretion of hippuric acid in the urine accounted for 66 to 95 per cent of the benzoic acid consumed as sodium benzoate. Small doses of sodium benzoate gave nearly perfect quantitative recovery of hippuric acid. As the amount of sodium benzoate was increased, the percentage recovered as hippuric acid decreased. It was assumed that this showed a lack of sufficient glycine in the animal for the detoxication of the benzoic acid. The addition of glycine

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<sup>1</sup> This study was a graduate research problem conducted under the direction and supervision of Professor T. S. Hamilton, Department of Animal Husbandry, University of Illinois.

to the diet containing sodium benzoate increased the growth rate and decreased the toxic effects.

Rittenberg and Schoenheimer (4) showed that animals use tissue glycine in preference to dietary glycine for the detoxication of benzoic acid. About two-thirds of the nitrogen in the hippuric acid excreted was not of dietary origin. An excess of dietary glycine did not change this ratio.

#### PLAN OF EXPERIMENT

White rats were fed diets containing calcium propionate, sodium propionate, sodium benzoate, zephiran, and sodium benzoate plus glycine. Feeding of the experimental diets started when the rats were about four weeks of age and continued for four to five weeks. The paired-feeding technique (2, 3) was used, with a modification so that one control animal was compared with two experimental animals. Eight triplicates (24 rats) were fed during each part of the experiment. In each triplicate, litter mates of the same sex were used and the maximum weight variation of the young rats at the start was 4 grams.

In series I the control animals were fed a basal diet containing calcium lactate, a second group was fed diets containing 1 per cent and 3 per cent calcium propionate, and a third group was fed diets containing 1 per cent and 3 per cent sodium propionate.

In series II the control diet contained calcium lactate, one experimental diet contained 3 per cent sodium benzoate, and the other experimental diet contained 3 per cent zephiran, a germicidal agent. Zephiran is sold as a ten per cent aqueous solution of a mixture of high molecular alkyl-dimethylbenzyl-ammonium chlorides. When used as a rinse for utensils, 1 part of zephiran is added to 5,000 to 10,000 parts of water.

In series III the control diet previously used was compared with an experimental diet containing 1 per cent sodium benzoate and another containing 3 per cent sodium benzoate plus glycine. Theoretically, .52 gram of glycine is required to detoxify 1 gram of sodium benzoate, but one gram of glycine was fed for each gram of sodium benzoate in order to supply more glycine than was needed.

In series IV all the rats used in series III were fed a diet containing 3 per cent sodium benzoate. The same grouping was maintained in order to study the residual effect of feeding small quantities of sodium benzoate and also of feeding glycine.

The composition of the diets used in each series is given in table 1. Waste by rats was prevented by mixing enough distilled water with the feed at feeding time to give the mixture the consistency of paste. Distilled water was supplied at all times in individual water fountains.

#### RESULTS

Comparisons of control rats with experimental rats showed that calcium

propionate, sodium propionate, and zephiran fed at the levels used in these trials did not decrease the amount of gain. Sodium benzoate fed at the rate of 3 per cent proved harmful: the amount of gain was much less than for the control group, and two rats out of eight died before the end of the trial.

TABLE 1  
*Composition of diets*

Series and group	Constituents of each diet					
	Fox chow	Yeast	Cod-liver oil	Salt	Calcium lactate	Experimental substance
	%	%	%	%	%	%
Series Ia:						
Control	91.5	5.0	2.0	0.5	1.0	
Ca propionate	91.5	5.0	2.0	0.5		1.0
Na propionate	91.5	5.0	2.0	0.5		1.0
Series Ib:*						
Control	89.5	5.0	2.0	0.5	3.0	
Ca propionate	89.5	5.0	2.0	0.5		3.0
Na propionate	89.5	5.0	2.0	0.5		3.0
Series II:						
Control	89.5	5.0	2.0	0.5	3.0	
Na benzoate	89.5	5.0	2.0	0.5		3.0
Zephiran	89.5	5.0	2.0	0.5		3.0
Series III:						
Control	89.5	5.0	2.0	0.5	3.0	
Na benzoate	91.5	5.0	2.0	0.5		1.0
Na benzoate plus glycine	89.5	5.0	2.0	0.5		3.0†
Series IV:†						
Na benzoate	89.5	5.0	2.0	0.5		3.0

\* Rats continued from series Ia.

† Rats continued from series III.

‡ One part of glycine was added for each part of sodium benzoate.

For the group of rats receiving 3 per cent sodium benzoate, the average amount of gain produced per 10 grams of feed consumed was 1.01 grams smaller than the average for the control group. This is considered a significant margin. The addition of glycine to the 3 per cent sodium benzoate diet reduced the toxic effect of the benzoic acid. The difference between the amount of gain produced per 10 grams of feed consumed when glycine was fed was only .24 gram in favor of the control group. This fact is probably significant, however, because the gain of the control individual over that of the rat receiving sodium benzoate plus glycine was consistent for all pairs tested. This result may indicate a limited capacity of the animal organism to synthesize glycine or to utilize dietary glycine for the detoxication of sodium benzoate.



TABLE 2  
Grams of gain produced

Series and groups	Gain by triplicates								Total gain	Average	Differ- ence†	S.D.	P. value	Odds	Gain/10 gms. feed								
	1		2		3		4									5		6		7		8	
Series Ia (4 wks.)																							
Control group	88	82	70	86	88	60	78	80	632	79.0						3.34							
Ca propionate	90	82	66	87	91	62	82	80	640	80.0	+ 1.0	2.29	.1429	1:7		3.38							
Na propionate	85	84	67	86	87	67	80	82	638	79.8	+ .75	2.11	.1881	1:5		3.37							
Series Ib (3 wks.)																							
Control group	50	26	30	50	43	20	33	36	288	36.0						1.74							
Ca propionate	52	16	34	50	39	17	36	35	279	34.9	- 1.12	4.25	.2586	1:4		1.68							
Na propionate	53	17	35	51	36	25	43	33	293	36.6	+ .63	6.08	.3994	1:3		1.77							
Series II (4 wks.)																							
Control group	63	70	65	74	69	65	74	77	577	69.6						3.24							
Na benzoate (3%)	51	53	58	*	*	59	32	32	285	47.5	- 21.5	15.98	.0148	1:68		2.23							
Zephiran (3%)	*	71	70	76	80	62	77	73	509	72.7	+ 2.1	4.67	.1525	1:7		3.35							
Series III (5 wks.)																							
Control group	82	100	98	103	97	100	98	98	776	97.0						3.12							
Na benzoate (1%)	87	96	98	97	95	97	97	102	769	96.1	- .88	3.55	.2666	1:4		3.09							
Na benzoate (3%) plus glycine	74	87	90	94	95	92	90	95	717	89.6	- 7.38	3.23	.0002	1:5000		2.88							
Series IV† (2 wks.)																							
Group 1	18	22	*	23	19	26	28	26	162	23.1						1.33							
Group 2	32	20	28	27	23	33	22	29	214	26.8	+ 3.43	5.90	.1033	1:10		1.55							
Group 3	32	24	29	*	25	32	30	22	194	27.7	+ 4.33	5.47	.0668	1:15		1.61							

\* Omitted because of incomplete results.

† Mean difference between control group and experimental group.

‡ Rats of group 1 continued from control group of series III.

Rats of group 2 continued from Na benzoate (1%) group of series III.

Rats of group 3 continued from Na benzoate (3%) plus glycine group of series III.

At the termination of the tests in series III, all the rats of that trial were changed to a diet containing 3 per cent sodium benzoate. Results obtained from this diet were compared with those of previous diets fed, but the probable error was large. The control group which had never received any sodium benzoate was most seriously affected by the change in diet. This group consumed 7.49 grams of feed per gram of gain compared to 6.44 for the group which had previously received 1 per cent sodium benzoate and 6.20 for the group which had previously received 3 per cent sodium benzoate plus glycine.

These results indicate that the group of rats which had previously received glycine had more resistance to the toxic effect of sodium benzoate than any other group. The utilization of stored glycine may explain the increased resistance. However, it seems reasonable to assume that the glycine reserve of group 2, which had received previously sodium benzoate and no glycine, should have been depleted and that additional quantities of sodium benzoate should have been very toxic. Instead, this group of rats made a greater gain than group 1 and practically as much gain as group 3. This fact may indicate that with limited feeding of sodium benzoate a mechanism was set up which enabled the animals to more readily combat toxicity.

Series II gives further evidence that rats which consume sodium benzoate develop a resistance to its harmful effects. The symptoms of toxicity were evident and the deaths caused by it occurred 7 to 20 days after the start of the trial. The rats which lived longer than 20 days showed an increasing resistance to the toxic effects of sodium benzoate.

The toxicity of sodium benzoate was expressed by definite, characteristic symptoms. The animal first became irritable and rigid, responding to the slightest touch or disturbance by biting the cage or the handler. As the symptoms progressed, it lost its power of coordinated movements and had convulsions.

A summary of the grams of gain produced in each part of this experiment is presented in table 2.

#### SUMMARY

A study was made of the amount of gain produced in growing white rats which were fed diets containing chemical agents used in food products or as a sterilizing agent. The amount of gain produced was used as an indication of the toxicity of these chemical agents. It is recognized that the method of measuring toxicity by the amount of gain produced has a limited application.

Neither calcium propionate nor sodium propionate, when fed in quantities up to 3.0 per cent of the diet, decreased the amount of gain.

Zephiran, a disinfectant made up of high molecular alkyldimethylbenzylammonium chlorides, when included in the diet at the rate of 3.0 per cent,

did not cause any ill effect. In fact, the zephiran group made slightly greater gain than the control group; and out of seven pairs of rats finishing the trial, five of the rats receiving zephiran made greater gains than their paired members in the control group. The results of this study indicated that zephiran is not toxic, and since it is a germicide there is a possibility that it could be employed to help control the bacterial flora of the alimentary canal in experiments where that feature is desired.

Sodium benzoate fed at the rate of 1.0 per cent in the diet did not affect the amount of gain. When the rate was increased to 3.0 per cent, definite toxic effects were observed. Numerous investigations have indicated that benzoic acid in the animal organism is detoxified by combining with glycine to form hippuric acid. It has been suggested that the animal organism has a readily available supply of glycine which can be used for the detoxication of benzoic acid. This study indicates that the animal organism has only a limited capacity for detoxifying benzoic acid in the body. The fact that addition of glycine to the sodium benzoate diet increased the amount of gain indicates a decrease in toxicity. The small difference between the gains produced in this group and the control group is considered significant, however, because individual comparisons showed that the control animals made greater gains than the experimental animals in every case.

The animal organism may develop a tolerance for sodium benzoate. Three groups of rats, which had received, respectively, no sodium benzoate, 1.0 per cent sodium benzoate, and 3.0 per cent sodium benzoate plus glycine, were all placed on a diet containing 3.0 per cent sodium benzoate. The group which had not previously received any sodium benzoate made the smallest gain and required the most food per gram of gain. The development of a tolerance for sodium benzoate was indicated by this fact and by the evidence obtained in series II that the toxic effects are expressed during the first 20 days of feeding.

The paired-feeding method of controlling food intake is believed essential to a study of this nature. If the food intakes of the control and experimental animals had not been equal, observed differences in amount of gain could have resulted from the difference in amount of food consumed.

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# DISTRIBUTION OF DIACETYL AND ACETYLMETHYLCARBINOL BETWEEN FAT AND WATER, WITH SPECIAL REFERENCE TO BUTTER<sup>1</sup>

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In butter made with butter culture, diacetyl and acetylmethylcarbinol are derived largely from the culture added to the cream and from the fermentation of citric acid during holding or ripening of the cream. Only small portions of these compounds present in the cream at churning appear in the finished butter. They are carried out in the buttermilk (2, 4, 5, 6, 8, 9, 15, 16, 17, 19) in relatively large amounts and also may be removed by the water used to wash the butter (6, 11, 13, 17).

Fat and water, the two constituents of butter which are present in large amounts, have very different solvent properties. The data reported herein deal with the distribution of diacetyl and acetylmethylcarbinol between fat and water and involve studies on (a) mixtures of "Wesson" oil and water or brine, (b) mixtures of butterfat and water or brine and (c) unsalted and salted butter.

## ANALYTICAL PROCEDURES

Diacetyl was determined by the colorimetric method of Prill and Hammer (18), with minor modifications used by Hoecker and Hammer (9). Color intensities were obtained with a Klett-Summerson photoelectric colorimeter. Acetylmethylcarbinol also was determined colorimetrically, using the procedure described by Hoecker and Hammer (9) for its estimation in butter.

## EXPERIMENTAL

### *Distribution of Diacetyl and Acetylmethylcarbinol in Mixtures of "Wesson" Oil and Water or Brine<sup>2</sup>*

The distribution of diacetyl between "Wesson" oil and water or brine was studied (a) by adding various amounts of an oil solution of diacetyl to 100 ml. portions of oil and then adding 100 ml. of either water or brine to each portion and (b) by adding various amounts of a water solution of diacetyl to 100 ml. portions of either water or brine and then adding 100 ml. of oil to each portion. The mixtures were held 2 days at room temperature in closed containers, with frequent shaking. The oil and water or brine were separated by centrifuging, the oil was pipetted off and diacetyl contents of

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<sup>2</sup> Brine regularly consisted of 14 gm. sodium chloride made up to 100 ml. with distilled water.

TABLE 1

*Distribution of diacetyl and acetylmethylcarbinol in mixtures of "Wesson" oil and water or brine*  
 Mixtures contained equal volumes of oil and water or brine and were held 2 days at room temperature

Series No.	Description of mixture	Diacetyl				Acetylmethylcarbinol			
		p.p.m. in mixture calc.*	p.p.m. in water	p.p.m. in oil	% in oil	p.p.m. in mixture calc.	p.p.m. in water	p.p.m. in oil	% in oil
1	0.5 ml.	1.86	2.93	0.78	21.0	4.62	8.45	0.79	0.86
	100 ml. oil + 1.0 ml. soln. A† or B‡ + 100 ml. water	3.54	5.75	1.33	18.8	8.79	16.70	0.88	0.51
	2.0 ml.	6.55	10.40	2.69	20.5	14.78	28.20	1.36	0.46
2	3.0 ml.	10.38	16.40	4.35	21.0	22.56	43.30	1.82	0.40
	0.5 ml.	1.41	1.98	0.83	29.5	3.87	7.04	0.69	0.89
	100 ml. oil + 1.0 ml. soln. A or B + 100 ml. brine	3.07	4.19	1.95	31.8	8.83	16.40	1.26	0.71
3	2.0 ml.	5.75	7.73	3.77	32.8	15.62	29.27	1.96	0.63
	3.0 ml.	9.34	12.50	6.18	33.1	23.10	43.13	3.07	0.66
4	0.5 ml.	1.85	3.00	0.70	18.9	3.92	6.87	0.96	0.12
	100 ml. water + 1.0 ml. soln. C§ or D   + 100 ml. oil	3.82	6.40	1.24	16.3	13.60	15.92	1.27	0.47
	2.0 ml.	7.78	12.92	2.64	17.0	16.71	31.80	1.62	0.48
4	3.0 ml.	12.31	21.00	3.62	14.7	26.11	49.90	2.32	0.45
	0.5 ml.	1.53	2.15	0.91	29.8	4.42	8.10	0.73	0.83
	100 ml. brine + 1.0 ml. soln. C or D + 100 ml. oil	3.31	4.71	1.91	28.9	7.90	14.70	1.10	0.70
	2.0 ml.	6.40	9.47	3.33	26.0	15.87	29.40	2.34	0.74
	3.0 ml.	9.75	14.70	4.80	24.6	24.27	46.20	2.33	0.48

\* Calculated from analyses of oil and water or brine.

† Soln. A = 100 ml. "Wesson" oil + 0.1 ml. diacetyl.

‡ Soln. B = 100 ml. "Wesson" oil + 0.2 gm. acetylmethylcarbinol.

§ Soln. C = 100 ml. water + 0.1 ml. diacetyl.

|| Soln. D = 100 ml. water + 0.2 gm. acetylmethylcarbinol.

the oil and water or brine were determined. In the studies on acetylmethylcarbinol the same general procedures were used. Table 1 gives the details of the trials and also the results of the analyses.

In the mixtures, the water or brine regularly contained higher concentrations of diacetyl and acetylmethylcarbinol than the oil, the differences being greater with acetylmethylcarbinol than with diacetyl. The percentage of diacetyl in a mixture that was contained in the oil was increased by sodium chloride in the water. The concentration of diacetyl in a mixture apparently did not affect the percentage in the oil. The concentrations of acetylmethylcarbinol in the oil were very low, and the different concentrations in the mixtures gave essentially the same percentage in the oil.

In the oil-water mixtures, concentrations of diacetyl and acetylmethylcarbinol in water ranged from 2.93 to 21.00 p.p.m. and from 6.87 to 49.90 p.p.m., respectively, and those in the oil varied from 0.70 to 4.35 p.p.m. and from 0.79 to 2.32 p.p.m., respectively. Of the total diacetyl and acetylmethylcarbinol in the mixtures, from 14.7 to 21.0 per cent and from 0.12 to 0.86 per cent, respectively, were in the oil.

In the oil-brine mixtures, concentrations of diacetyl and acetylmethylcarbinol in the brine ranged from 1.98 to 14.70 p.p.m. and from 7.04 to 46.20 p.p.m., respectively, while those in the oil varied from 0.83 to 6.18 p.p.m. and from 0.69 to 3.07 p.p.m., respectively. The percentages of the total diacetyl and acetylmethylcarbinol in the mixtures that were in the oil ranged from 24.6 to 33.1 and from 0.48 to 0.89, respectively.

A series of oil-water mixtures, each containing 200 gm. of "Wesson" oil and 40 gm. of water, were prepared and different amounts of either diacetyl or acetylmethylcarbinol added. Additions of the various concentrations of diacetyl or acetylmethylcarbinol were made by using equal amounts of the compounds as oil and water solutions. The mixtures were then treated the same as those involving equal volumes of oil and water or brine. Data on the distribution of diacetyl and acetylmethylcarbinol in the mixtures are given in table 2.

As in the previous trials, the water regularly contained higher concentrations of diacetyl and acetylmethylcarbinol than the oil, and the differences were greater with the carbinol than with diacetyl. With both diacetyl and acetylmethylcarbinol, the percentages in the mixtures that were contained in the oil varied considerably, but with each compound the highest value was obtained with the lowest concentration.

In the mixtures, concentrations of diacetyl and acetylmethylcarbinol in the water ranged from 0.53 to 6.90 p.p.m. and from 10.92 to 118.00 p.p.m., respectively, while those in the oil varied from 0.24 to 2.19 p.p.m. and from 0.87 to 5.43 p.p.m., respectively. Of the total diacetyl and acetylmethylcarbinol in the mixtures, from 56.5 to 69.0 per cent and from 18.5 to 28.7 per cent, respectively, were in the oil.

TABLE 2

*Distribution of diacetyl and acetylmethylcarbinol in mixtures of "Wesson" oil and water*  
 Mixtures contained 200 gm. oil and 40 gm. water and were held 2 days at room temperature

Trial No.	Diacetyl				Acetylmethylcarbinol			
	p.p.m. in mixture calc.*	p.p.m. in water	p.p.m. in oil	% in oil	p.p.m. in mixture calc.	p.p.m. in water	p.p.m. in oil	% in oil
1	0.29	0.53	0.24	69.0	2.54	10.92	0.87	28.7
2	0.47	0.96	0.37	66.0	4.00	19.00	1.01	21.0
3	0.68	1.53	0.52	63.3	5.65	26.10	1.57	23.2
4	0.70	1.60	0.52	61.4	6.49	30.70	1.67	21.4
5	1.09	2.43	0.83	63.3	11.33	54.40	2.75	20.2
6	1.68	4.40	1.14	56.5	13.04	64.00	2.89	18.5
7	2.97	6.90	2.19	61.3	24.13	118.00	5.43	18.8

\* Calculated from analyses of oil and water.

With about the same concentration of diacetyl or acetylmethylcarbinol in mixtures of equal volumes of oil and water and in mixtures of 200 gm. oil and 40 gm. water, the concentrations of the compounds in both the water and the oil were higher in the latter mixtures. Since in these mixtures the oil comprised such a large portion of the total weight, it contained larger percentages of the diacetyl and acetylmethylcarbinol than in mixtures of equal volumes of oil and water.

*Distribution of Diacetyl and Acetylmethylcarbinol in Mixtures of Butterfat and Water or Brine*

In studying the distribution of diacetyl between butterfat and water or brine, butterfat was obtained by melting sweet cream butter (made without butter culture), allowing the fat and serum to separate at 45° C. and then decanting and filtering the fat at 45° C. Mixtures of 84 per cent melted fat and 16 per cent water or brine were placed in quart jars and various amounts of diacetyl added, the additions consisting of equal amounts of diacetyl as "Wesson" oil and water solutions. The jars were closed and placed in a small experimental churn where the mixtures were agitated for 1 hour. After holding the mixtures at 4° C. for various periods, the fat and water or brine were separated by placing the mixtures at 45° C. until the fat had melted, centrifuging and then pipetting off the fat. Diacetyl concentrations in the fat and the water or brine were determined. In the trials with acetylmethylcarbinol, the same procedures were employed. Table 3 gives the data on one trial which is representative of the five carried out.

The diacetyl and acetylmethylcarbinol showed the same general distribution as in the mixtures involving "Wesson" oil, with the water or brine regularly containing higher concentrations of the compounds than the fat and the differences being greater with acetylmethylcarbinol than with diacetyl. Sodium chloride increased the percentages of the compounds in the

TABLE 3

*Distribution of diacetyl and acetylmethylcarbinol in mixtures of butterfat and water or brine*

Mixtures contained 84 per cent butterfat and 16 per cent water or brine and were held 7 and 30 days at 4° C.

Description of mixture	Days held at 4° C.	Diacetyl				Acetylmethylcarbinol			
		p.p.m. in mixture calc.*	p.p.m. in water	p.p.m. in oil	% in oil	p.p.m. in mixture calc.	p.p.m. in water	p.p.m. in oil	% in oil
Butterfat-water	7	1.11	2.23	0.90	68.0	2.81	12.50	0.97	28.9
		2.25	4.90	1.75	65.3	4.85	22.30	1.52	26.4
		4.06	8.60	3.19	66.0	8.34	38.00	2.70	27.1
		4.78	10.91	3.61	63.4				
		6.15	13.59	4.74	64.7	12.81	53.00	5.15	33.7
		7.50	17.44	5.61	62.9	20.56	97.58	5.89	24.0
	30	0.86	1.87	0.67	65.2				
		1.58	3.50	1.21	64.6	5.14	22.00	1.93	31.6
		3.38	7.60	2.56	63.7	8.67	40.00	2.71	26.2
		3.60	7.60	2.83	66.2	10.81	45.00	4.30	33.4
		4.93	10.60	3.84	65.5	14.83	65.50	5.27	29.8
		5.04	10.30	4.04	65.4	19.76	93.60	5.89	24.5
Butterfat-brine	7	0.81	1.08	0.76	78.7	3.31	10.00	2.04	51.7
		1.40	1.90	1.31	78.4	5.13	20.30	2.24	36.7
		2.67	3.92	2.43	76.5	8.85	35.00	3.88	36.7
		3.46	5.04	3.16	76.7	12.94	54.20	5.10	33.1
		5.07	8.08	4.50	74.5	18.70	77.60	7.48	33.6
		6.01	9.12	5.42	75.7	21.75	89.20	8.88	34.3
	30	0.62	0.80	0.59	79.0	3.34	11.80	1.73	43.4
		1.25	1.75	1.15	78.4	5.21	20.40	2.33	37.4
		2.14	3.11	1.95	75.3	9.47	37.30	4.16	37.0
		3.25	5.11	2.89	74.7	13.62	56.10	5.54	34.1
		3.85	6.46	3.35	73.3	18.66	76.70	7.60	34.2
		5.81	8.78	5.23	75.7	22.24	91.60	9.00	34.0

\* Calculated from analyses of fat and water or brine.

mixtures that were contained in the fat. Mixtures held 7 days and 30 days showed essentially the same distribution of diacetyl and the carbinol.

In the butterfat-water mixtures, concentrations of diacetyl and acetylmethylcarbinol in the water ranged from 1.87 to 17.44 p.p.m. and from 12.50 to 97.58 p.p.m., respectively, and those in the fat varied from 0.67 to 5.61 p.p.m. and from 0.97 to 5.89 p.p.m., respectively. The fat contained from 62.9 to 68.0 per cent of the diacetyl and from 24.0 to 33.7 per cent of the acetylmethylcarbinol in the mixtures.

In the butterfat-brine mixtures, concentrations of diacetyl and acetylmethylcarbinol in the brine ranged from 0.80 to 9.12 p.p.m. and from 10.00 to 91.60 p.p.m., respectively, while those in the fat varied from 0.59 to 5.42 p.p.m. and from 1.73 to 9.00 p.p.m., respectively. The fat contained from 73.3 to 79.0 per cent of the diacetyl and from 33.1 to 51.7 per cent of the acetylmethylcarbinol in the mixtures.



The results on the five trials with butterfat and water or brine are summarized in table 4, the data being divided on the basis of the concentrations of diacetyl or acetylmethylcarbinol in the mixtures.

TABLE 4

*Summary of the distribution of diacetyl and acetylmethylcarbinol in all the butterfat and water or brine mixtures*

Mixtures contained 84 per cent butterfat and 16 per cent water or brine and were held various periods at 4° C.

Description of mixtures and groupings	p.p.m. in mixture calc.*	Number of mixtures	Average p.p.m. in		Average % in fat
			Water or brine	Fat	
Diacetyl					
Butterfat-water mixtures					
Group 1 . . . . .	0 to 1.50	13	1.60	0.64	71.8
Group 2 . . . . .	1.51 to 5.00	21	6.45	2.60	69.2
Group 3 . . . . .	above 5.00	17	12.01	5.85	71.5
Butterfat-brine mixtures					
Group 1 . . . . .	0 to 1.50	11	1.23	0.95	80.6
Group 2 . . . . .	1.51 to 5.00	18	4.08	2.96	78.9
Group 3 . . . . .	above 5.00	23	12.96	9.54	79.0
Acetylmethylcarbinol					
Butterfat-water mixtures					
Group 1 . . . . .	0 to 8.00	13	23.46	1.90	30.0
Group 2 . . . . .	8.01 to 24.00	18	73.86	4.93	26.4
Group 3 . . . . .	above 24.00	18	185.17	12.13	22.9
Butterfat-brine mixtures					
Group 1 . . . . .	0 to 8.00	13	20.26	2.47	40.3
Group 2 . . . . .	8.01 to 24.00	20	63.68	6.41	34.9
Group 3 . . . . .	above 24.00	18	149.30	14.54	33.9

\* Calculated from analyses of fat and water or brine.

The average values in the table support the various points already noted. They also show that as the concentration of diacetyl in a mixture increased, the percentage in the fat did not change appreciably, while as the concentration of acetylmethylcarbinol in a mixture increased, the percentage in the fat decreased. These relationships are suggested by the data on the individual mixtures, but irregularities in the results tend to obscure them.

#### *Distribution of Diacetyl and Acetylmethylcarbinol in Unsalted and Salted Butter*

In the studies on the distribution of diacetyl and acetylmethylcarbinol between the fat and serum of unsalted and salted butter, the serum was separated from the fat (a) by subjecting chilled butter to high pressures and (b) by centrifuging melted butter. The unsalted butter was of the high-flavor type, being churned from cream that had been ripened with a butter culture to a relatively high acidity. The salted butter either was

churned from sweet cream held over night at about 4° C. after addition of 8 per cent butter culture or was churned from sweet cream (containing no culture) with addition of a solution of diacetyl or a distillate of butter culture during working of the butter.

For the separation of the serum and fat by pressing, the butter was held at approximately -18° C. for 24 hours, after which it was shredded and 400 gm. mixed with 900 gm. of fine sand. The mixture was then subjected to approximately 1200 lbs. pressure per square inch in a hydraulic press (12). The sand and the parts of the press coming in contact with the mixture were cooled to approximately 4° C. before use so that the temperature of the butter would not increase too rapidly. With the procedure employed, a portion of the butter serum was obtained. Also, a small amount of the mixture came through the filter cloth; this material was held at 45° C. and the fat recovered by centrifuging and pipetting. For the separation of the serum and fat by melting, the butter was held in a closed container at 45° C. until the fat had melted, and the mixture was then centrifuged and the fat pipetted off.

Diacetyl and acetylmethylcarbinol determinations were made on the original butter and on the fat and serum. The percentages of fat and water in the butter were determined by the Mojonnier method, and from these values and the analyses on the fat and serum the amounts of diacetyl and acetylmethylcarbinol in the butter were also calculated. Table 5 gives the data.

As with the mixtures of butterfat and water or brine, the serum of butter contained higher concentrations of diacetyl and acetylmethylcarbinol than the fat, with the greatest differences again involving the carbinol. The averages of the percentages of diacetyl and acetylmethylcarbinol contained in the fat were smaller in unsalted than in salted butter. The percentage of diacetyl contained in the fat apparently was independent of the concentration in the butter, whereas the percentage of acetylmethylcarbinol contained in the fat usually decreased somewhat as the concentration in the butter increased. Addition of a solution of diacetyl or a distillate of butter culture to salted butter resulted in essentially the same distribution of diacetyl as when the butter was made from cream containing butter culture. When the serum was separated by pressing the butter, the fat usually contained slightly higher percentages of diacetyl and acetylmethylcarbinol than when the serum was separated by melting the butter. The amounts of diacetyl and acetylmethylcarbinol in the butter, as calculated from its composition and analyses of the fat and serum, agree fairly closely with the determined amounts.

In the unsalted butter, diacetyl and acetylmethylcarbinol concentrations in the serum ranged from 0.25 to 3.55 p.p.m. and from 4.85 to 119.16 p.p.m., respectively, and those in the fat varied from 0.19 to 1.11 p.p.m. and from

TABLE 5  
Distribution of diacetyl and acetylmethylcarbinol in unsalted and salted butter

Sample No.	Serum separated by	Diacetyl				Acetylmethylcarbinol					
		p.p.m. in butter		p.p.m. in serum	p.p.m. in fat	% in fat	p.p.m. in butter		p.p.m. in serum	p.p.m. in fat	% in fat
		detr.*	calc.†				detr.	calc.			
Unsalted											
1	pressing	1.16	0.96	2.88	0.61	53.2	19.75	17.41	78.65	6.20	29.8
2	pressing pressing†	0.92 0.24	0.81 0.20	2.27 0.25	0.45 0.19	44.4 75.0					
3	pressing melting	0.57 0.57	0.58 0.50	1.79 1.54	0.33 0.35	46.6 58.0	1.58 1.38	1.60 1.56	5.08 4.85	0.89 0.98	45.6 46.8
4	pressing melting	1.29 1.29	1.09 1.11	3.13 2.34	0.72 0.89	55.0 67.5	23.20 23.20	23.05 20.44	119.16 96.00	5.46 6.61	19.5 27.1
5	pressing melting	1.42 1.42	1.39 1.33	3.55 2.50	1.04 1.11	62.1 69.4	20.90 20.90	20.25 20.08	92.40 88.90	6.40 6.86	26.2 28.2
6	pressing melting	0.99 0.99	0.91 0.94	2.70 1.75	0.58 0.79	52.7 70.3	10.10 10.10	7.94 8.34	32.00 33.50	3.40 3.60	35.6 35.9
Salted											
7	pressing	0.16	0.19	0.44	0.14	63.2	4.56	4.23	18.53	1.76	34.2
8	pressing	0.08	0.09	0.20	0.07	66.1	3.18	2.69	12.30	0.91	27.5
9	pressing	0.11	0.11	0.26	0.09	61.3	3.00	3.03	13.00	1.09	29.0
10	pressing melting	0.10 0.10	0.09 0.09	0.21 0.14	0.08 0.09	63.2 78.4	2.40 2.40	2.69 2.58	11.00 8.66	1.04 1.25	30.8 43.4
11	pressing melting	0.05 0.05	0.03 0.03	0.07 0.11	0.02 0.02	65.5 48.5	0.54 0.54	0.50 0.54	1.72 1.93	0.29 0.30	46.0 44.4
12	pressing melting	0.69 0.69	0.71 0.73	1.55 1.15	0.55 0.66	61.8 72.3	13.85 13.85	13.50 11.83	59.00 45.40	4.00 4.87	23.6 32.8
13	pressing melting‡	0.92 0.92	0.96 0.97	2.00 1.33	0.76 0.92	61.6 74.6					
14	pressing melting	0.50 0.50	0.44 0.52	0.73 0.80	0.39 0.48	70.5 72.8					

\* Determined.

† Calculated on basis of composition of butter and analyses of serum and fat.

‡ Butter held 2 weeks at 4° C.

§ Solution of diacetyl added to sweet cream butter.

|| Distillate of butter culture added to sweet cream butter.

0.88 to 6.86 p.p.m., respectively. The fat contained from 44.4 to 75.0 per cent of the diacetyl and from 26.2 to 46.8 per cent of the acetylmethylcarbinol in the butter.

In the salted butter, diacetyl and acetylmethylcarbinol concentrations in the serum ranged from 0.07 to 2.00 p.p.m. and from 1.72 to 59.00 p.p.m., respectively, while those in the fat varied from 0.02 to 0.92 p.p.m. and from 0.29 to 4.87 p.p.m., respectively. Of the total diacetyl and acetylmethylcarbinol in the butter, the fat contained from 48.5 to 78.4 per cent and from 23.6 to 46.0 per cent, respectively.

#### DISCUSSION OF RESULTS

The presence of diacetyl and acetylmethylcarbinol in the fat of butter and of mixtures of fat and water or brine is in agreement with various investigations (3, 7, 8, 10, 15, 17). However, Makar'in (13, 14) found diacetyl (a trace) in the fat of only one of eight samples of butter, whereas the serum always contained diacetyl. He believed that the aroma of butter is influenced largely by the concentration of the aqueous constituents of the cream that are retained in the butter. On the basis of averages of the percentages of diacetyl and acetylmethylcarbinol in cream that were retained in butter, Barnicoat (1, 2) suggested that these compounds are present only in the serum. If this were the case, the percentages of the compounds in the cream that are retained in the butter should be the same, and actually a higher percentage of diacetyl than of acetylmethylcarbinol is retained (19).

Since large percentages of the diacetyl and acetylmethylcarbinol in cream at churning are removed with the buttermilk, higher concentrations of the compounds would be expected in the serum than in the fat of butter. Although the concentrations in the fat are relatively low, the percentages of the compounds in butter that are contained in the fat are comparatively high because butter contains approximately 80 per cent fat.

The difference in the partitioning of diacetyl and acetylmethylcarbinol between fat and serum of butter (or between fat and water or brine in the mixtures) is what would be expected from the chemical constitution of the compounds. Davies (8) stated that since the vapor pressure of diacetyl is much higher than that of acetylmethylcarbinol, a greater portion of diacetyl than of the carbinol would be expected in the fat. Apparently, the partitioning of diacetyl and carbinol between the serum and fat in butter reaches an equilibrium in a relatively short time.

The distribution of diacetyl in butter into which a solution of diacetyl or a distillate of butter culture has been worked should be essentially the same as in butter made from cream containing butter culture; diacetyl is produced by the organisms in the serum of cream (or butter) and therefore in both cases the partitioning of the diacetyl is from the serum to the fat.

The greater percentage of diacetyl and acetylmethylcarbinol in the fat

in mixtures of fat and brine than in mixtures of fat and water presumably is due to the salting-out effect of the sodium chloride. Some of the older procedures for distilling diacetyl from solutions made use of this effect through the addition of sodium chloride to a solution before distillation.

Comparative solubilities in fat probably explain why with diacetyl the concentration in butter does not affect the percentage of the total that is retained in the fat, while with acetylmethylcarbinol an increase in the concentration in butter decreases the percentage of the total that is retained in the fat. The low solubility of acetylmethylcarbinol soon limits the amount taken up by the fat.

The variations among samples of butter in the percentages of the total diacetyl or acetylmethylcarbinol that is contained in the fat probably are due to several factors, such as the composition of the butter, the physical state of the fat, the churning procedure and the degree to which water is dispersed in the butter. Also, analytical errors involved in determining very small quantities of the compounds may be of minor significance.

#### SUMMARY

In unsalted and salted butter, both the serum and the fat contained diacetyl and also acetylmethylcarbinol. The serum contained higher concentrations of the compounds than the fat, the differences being greater with acetylmethylcarbinol than with diacetyl. In each type of butter, a larger percentage of the total diacetyl than of the total acetylmethylcarbinol was contained in the fat. Butter into which a solution of diacetyl or a distillate of butter culture had been worked showed the same general distribution of diacetyl as butter made from cream containing butter culture.

In general, the data obtained on mixtures of "Wesson" oil and water or brine and mixtures of butterfat and water or brine agree with the results obtained on butter. In such mixtures, and also in butter although the results were not as definite as with the mixtures, the addition of sodium chloride increased the percentage of diacetyl or acetylmethylcarbinol that was in the fat. The concentration of diacetyl in the mixtures or in butter apparently did not affect the percentage contained in the fat, but as the concentration of acetylmethylcarbinol increased the percentage contained in the fat decreased. Mixtures of butter fat and water or brine held at 4° C. showed essentially the same distribution of diacetyl and acetylmethylcarbinol after 30 days as after 7 days.

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## GREETINGS FROM YOUR PRESIDENT

Certainly those of us who are considered too old to shoulder a rifle must consider ourselves fortunate to be associated with an industry so vital to the well-being of both soldier and civilian and hence to the defense of our country. Never was there a time when so great a service can be rendered by the dairy industry. "She who gives the most is the most highly esteemed" can well be said of the dairy cow upon whom we depend for our livelihood. Isn't this axiom a good one for us all to keep in mind in the days of toil that lie ahead?

It seems to me that the American Dairy Science Association is unique in that it has a membership that works together and members who give willingly and unstintingly of their time and talent by working on various committees. The response to my recent committee appointments has been splendid and is very much appreciated. I am sure it presages a successful year and a banner meeting in East Lansing next June.

Speaking of our annual meeting, I am sure we all appreciate the cordial invitation of President Hannah of Michigan State College to visit the campus of that University the week of June 21, 1942. While "business and education as usual" may not be possible in this war torn world we must keep on "operating the milking machine" and learning all we can about how to make the best use of the product. Let us, therefore, make our plans to attend our annual inspiration meeting in June. Professor Weaver and his staff have their plans for our entertainment well under way and the program committee assures us of the best program yet. The symposia, started last year, will continue to be a feature and it is hoped that this feature may help draw an ever increasing number to our meetings from the commercial field.

Don't forget to tell the girls and kiddies about this meeting. They will see that you go. The fact that the social side of our meetings seems like a big family party appeals to all, I am sure.

Just a closing word to our members who are wearing the uniform of Uncle Sam or who may have to don it before our meeting. Rest assured we are pulling for you. Keep the old chin up and keep your membership up. There will be times when you will want to see what the Journal has to say, and that, with the maturing influence of the Service will make you a valuable asset to the industry when you can change the khaki or blue for the white uniform once more.

H. F. JUDKINS





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## THE DANGER OF HYDROCHLORIC ACID GAS POISONING WHEN TESTING SALT-TREATED CREAM

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### INTRODUCTION

Several investigators have reported the use of common salt in cream in fairly high concentrations (5 to 13 per cent) in order to delay souring during storage on the farm or while on long shipments and thus maintain a better quality of cream for butter and ice cream manufacture.

### REVIEW OF LITERATURE

A patent was granted to O. E. Williams (1) of the United States Department of Agriculture on January 3, 1939, for a new method of preserving cream by the use of sodium chloride. By this method, it was claimed, cream held at room temperature for a considerable length of time would make as high scoring butter as fresh cream.

Thompson and Macy (5) in a study of the "Effect of Salt on the Microflora and Acidity of Cream" using 5.0, 7.5, and 10.0 per cent sodium chloride found that ". . . in general, the results indicate, with increasing salt concentrations in cream, the growth of bacteria and especially yeast was quite effectively checked if the cream was maintained at reasonably low temperatures and as a consequence the acidity of the cream did not increase materially during the 10-day period of storage."

Caulfield, Nelson, and Martin (3) found that ". . . the amount of salt necessary to effectively prevent deterioration in cream was dependent upon the time and temperature of storage. The addition of 13 per cent salt to cream held at 70° F. for three or more days before the salt was added did not prevent further deterioration of the cream." This limited the method largely to farm use. They suggested that the possible effects of prolonged exposure of the metallic cream containers to salted cream and the corrosive action of the brine on the can, which might shorten the life of the can and

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damage the flavor and keeping quality of the cream and of the resulting butter, be given further consideration. A modified Babcock test for butter-fat was given because of foaming of the salted cream and the formation of a grayish-brown deposit at the base of the fat column which interfered with the reading. This modified test was more time-consuming than the conventional Babcock test.

Castell and Garrard (2) found that butter produced from salted cream stored for 8 days at 60 to 77° F. was superior to butter made from unsalted cream stored at 50° F. for 8 days. They stated that fresh cream containing 7 per cent salt could be held in satisfactory condition for 8 days at 77° F.

#### EXPERIMENTAL

Samples of sodium-chloride-treated cream were analyzed according to a modified method developed at the University of Idaho Agricultural Experiment Station (6) which would determine the kind and amounts of gasses released, whether chlorine or hydrochloric acid gas. The first tests were made upon a freshly prepared cream-salt solution of 9.09 per cent sodium chloride. The results are shown in figure 1.

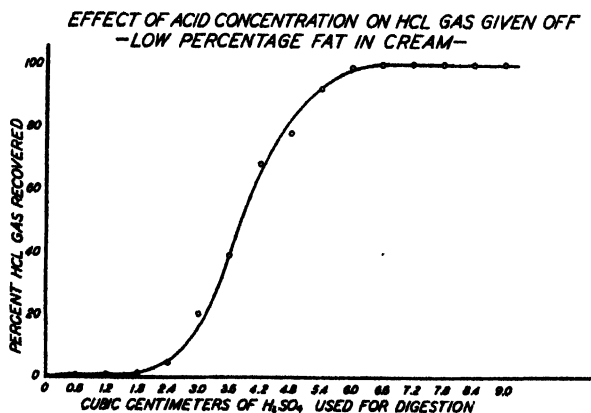


FIG. 1.

It is evident from figure 1 that in 30 per cent cream the gas given off is hydrochloric acid gas and that it is entirely released from 9 grams of cream by as little as 6.6 cc. of concentrated sulfuric acid. With higher percentages of fat in the cream it was found necessary to add to the cream up to and including an equal volume of water in order to release all the hydrochloric acid gas. This is shown in figures 2 and 3.

Apparently with high percentage cream there is either some absorption of the sodium chloride by the fat or else there is not sufficient water in the sample to hold all the salt in solution. When more water is added the hydrochloric acid gas is completely released.

## DISCUSSION

Samples of cream received from individual farmers must be tested for fat content in order that the correct price may be given for their product. When the samples were tested by the Babcock method the sulfuric acid reacted with the sodium chloride not only to produce excessive foaming but also to release relatively large amounts of hydrochloric acid gas at a concentration which might cause dangerous accumulations in the air of small poorly ventilated laboratories such as are often found in many creameries.

*EFFECT OF SHAKING AND STANDING ON SAMPLES  
—HIGH PERCENTAGE FAT IN CREAM—*

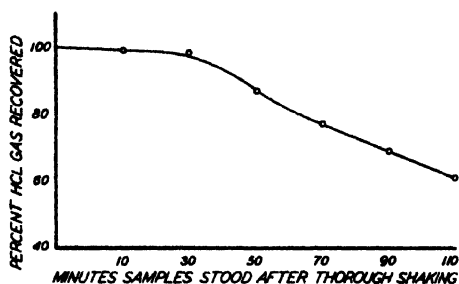


FIG. 2.

*EFFECT OF WATER ADDITIONS  
TO ABOVE CREAM*

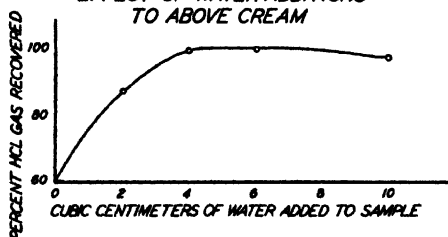


FIG. 3.

Hydrochloric acid gas is an irritant which, because it is readily soluble, acts primarily upon the upper respiratory tract. As little as 2,000 to 5,000 parts per million of the gas in air will produce clouding of the cornea and, after an hour, violent inflammation of the lining of the nose and throat and congestion and hemorrhages in the lungs. With only one-tenth of the above concentrations bronchial catarrh can be produced. Henderson and Haggard (4) give the following:

Physiological responses to various concentrations of hydrochloric acid gas	HCl gas in p.p.m. of air
Maximum concentration allowable for prolonged exposure	10
Maximum concentration allowable for $\frac{1}{2}$ to 1 hour exposure	50
Dangerous for even short exposure	1,000-2,000

Calculating the amounts of hydrochloric acid gas released from various salt concentrations in cream we find that a 13 per cent salted cream will release 0.7920 gram of HCl or approximately 487 cc. of gas while a 10 per cent salted cream will release 0.5612 gram or about 345 cc. of gas. Likewise, 7.5 per cent will release 0.4209 gram or 260 cc. and 5 per cent 0.2806 gram or 173 cc. of HCl gas.

TABLE 1

*Hydrochloric acid gas allowable compared with gas released from salted cream*

HCl gas allowable according to Henderson and Haggard (4)		Idaho experimental data		
	HCl gas in p.p.m. of air	Concentration of salted cream	Diffusion of HCl gas from 1 sample in room of 1000 cu. ft. capac.	Diffusion of HCl gas from 24 samples in room of 1000 cu. ft. capac.
		<i>per cent</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
Maximum allowable for prolonged exposure	10	13.0	17.2	413
Maximum allowable for $\frac{1}{2}$ to 1 hour exposure	50	10.0	12.2	292
Dangerous for even short exposures	1000-2000	7.5	9.15	220
		5.0	6.1	146

Assuming a laboratory of 1,000 cubic feet capacity, we find, as shown in table 1, that a single sample of salted cream, even if completely diffused, will cause a concentration of 17.2 p.p.m. in the air from a 13 per cent solution; 12.2 p.p.m. from a 10 per cent solution; 9.15 p.p.m. from a 7.5 per cent solution; and 6.1 p.p.m. from a 5 per cent solution. Since the maximum concentration allowable for prolonged exposure is 10 p.p.m., the release of hydrochloric acid gas from even one sample is dangerous.

Several conditions increase this danger. First, hydrochloric acid gas has a specific gravity of 1.278 when air equals 1.000. Thus the rate of diffusion is slow and the gas does not rise rapidly but remains in heavy concentration on the table in the immediate vicinity of the operator. Second, the operator usually does not make a single determination but rather runs them in sets of 12 or 24 at a time and, in the larger creameries, several times a day. Should the samples of salted cream be segregated and tested separately a set of 24 samples of 13 per cent salted cream would cause a concentration of approximately 413 p.p.m. of gas in a room of 1,000 cubic feet capacity if completely diffused; 293 p.p.m. from the 10 per cent salted cream, and equivalent amounts from the lower concentrations. This would cause dangerous exposures even for short lengths of time and when several sets of determinations are run each day, one can readily understand the increasing danger. Third, many times the testing laboratory does not have a capacity

of 1,000 cubic feet but is smaller, thus increasing the concentration of the gas. Fourth, even though the laboratory were large the slow rate of diffusion would cause high concentrations of the gas and the necessity for the operator to work over a large set of samples for a considerable period of time would be an extremely dangerous practice.

Caulfield, Nelson and Martin (3) have given a modified method for the Babcock test to minimize the effect of foaming by adding an equal volume of water to the sample and then adding the sulfuric acid in three equal volumes with 10 and 5 minute intervals between additions. As shown in figure 3, the addition of an equal volume of water to the cream causes a complete release of the hydrochloric acid gas. The longer standing increases the time the operator must be in contact with the fumes and thus increases the danger of injury.

The installation of well ventilated hoods has been suggested for the creamery laboratory so that the gas fumes will be carried away as fast as formed. Although this is expensive, some such procedure must be followed if the practice of salting cream is to come into general use. The smallness of many laboratories as well as the cost of equipment will make such installation difficult and expensive and it is questionable whether it would be done.

Even with installed hoods, there is always the ever constant possibility that the liberated fumes of hydrochloric acid gas may be breathed at any time while testing for butter-fat content with resultant danger to throat and lungs. The operator is not always aware of the destroying action of these fumes and may become affected by them before realizing their harm.

#### CONCLUSIONS

1. Salted creams of 5 to 13 per cent concentrations, when treated with sulfuric acid for the determination of fat content by the Babcock method, release the fumes of hydrochloric acid gas which are dangerous to health.

2. Single samples of salted cream of 7.5, 10.0, and 13 per cent concentrations release hydrochloric acid gas in amounts above the maximum allowable for prolonged exposure.

3. Sets of 12 to 24 samples in any of the concentrations tested, released hydrochloric acid gas above the maximum allowable for even short exposure ( $\frac{1}{2}$  to 1 hour).

4. The slow rate of diffusion causes high concentrations of gas in the immediate vicinity of the operator, thus increasing the danger of injury.

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# EFFECT OF HOLDING CREAM IN THE BUYING STATION UPON THE MOLD CONTENT AND CERTAIN OTHER QUALITY FACTORS<sup>1</sup>

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The activities of the Federal Food and Drug Administration in condemning butter containing excessive amounts of mold as determined by the Wildman microscopic method (9) have encouraged the use of the Parsons visual mold test (6) as a means of detecting unfit cream. This extended usage of the visual mold test has made it desirable to have more information concerning the conditions under which the test may be used as a reliable indication of the fitness of cream for buttermaking.

The amount of mold in cream has been shown to be an indication of the conditions under which the cream has been produced and handled on the farm (1, 3, 4, 6, 7, 8). Many creameries are now using the visual mold test on cream which has been collected in cream buying stations and held for varying periods of time before shipment to the central plants. Parfitt and Galema (5) have reported that holding cream in airtight containers for 72 hours at temperatures of 80° to 90° F. resulted in a marked reduction in mold content compared to cream held in open containers. The cream held in closed containers was found to be more desirable in flavor and to make a better flavored butter than would cream held with free access to air. They also stated that such cream showed less protein decomposition. More information is needed concerning the value of the visual mold test as a measure of the quality of cream after it has been held without adequate refrigeration in the buying station. The studies herein reported were designed to obtain more information regarding changes which occur in cream held in a representative cream station.

## METHODS

This investigation was conducted during the month of May, 1941. During the period in which cream samples were collected air temperature usually was in the high 80's and occasionally in the low 90's, and the minimum temperature each day usually was in the low 60's and occasionally in the high 50's. In the station under observation, cream was held in 10-gallon cans in a small, partially insulated room kept about 10° F. below atmospheric temperature by the use of a small fan to blow air over a small piece of ice. The cream in each can usually was a mixture from several farms.

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<sup>1</sup> Contribution No. 139, Department of Dairy Husbandry and contribution No. 208, Department of Bacteriology.



Samples were taken from each can at the close of buying activities for the day, and again from the same cans immediately before they were shipped to the creamery. The cream was thoroughly agitated and the samples taken in sterile jars. The samples were immediately cooled and held at 40° F., or below, until analyses could be made. The temperature of the cream and the amount of air space between the surface of the cream and the closed lid were observed in each case after the samples had been secured.

The acidity of each cream sample was determined by titrating a 9.0 gram sample, plus 9.0 milliliters of distilled water, with tenth-normal sodium hydroxide, using phenolphthalein as the indicator. The results were calculated as per cent lactic acid. The mold content of the cream was estimated by two methods, the Parsons modification (6) of the Wildman methylene-blue-borax visual mold test (9), and the plate count method using acidified potato dextrose agar according to the procedure outlined in Standard Methods for Examination of Dairy Products (2). Yeasts and molds were counted separately.

The standards used for evaluating the results of the visual mold tests were somewhat different from those proposed by the American Butter Institute, seven standards being employed instead of four, thus making more detailed classification possible. A comparison of the American Butter Institute standards and those used in this study is made in table 1. The cream samples also were graded organoleptically by two or more judges.

TABLE 1

*Comparison of visual mold standards used in this study with those proposed by the American Butter Institute*

Class	Standards used in this study	Corresponding American Butter Institute Standards
Good . . . . .	1	1
	2	
Fair . . . . .	3	2
Doubtful . . . . .	4	3
Excessive . . . . .	5	4
	6	
	7	

## RESULTS

In the first part of this study 75 cans of cream were examined before and after holding either one or two days in the station. During the holding period the average visual mold score increased only 0.03 units, indicating that, for the entire series, no significant change in mold score had occurred. The titratable acidities were determined on only 46 of these lots of cream and an average increase of 0.15 per cent was noted. These results indicate that the cream may change appreciably in other respects while the visual mold remains essentially constant.

*Changes taking place during holding of cream in a cream buying station*

Sample No.	Days held	Temperature		Grade		Per cent titratable acidity		Mold count—plate method		Yeast count—plate method		Visual mold score	
		In ° F.	Out ° F.	In	Out	In	Out	Thousands per ml.	Per cent change	Thousands per ml.	Per cent change	In	Out
1	2	70		2	2	0.62		115		850		3	2
2	2	70	75	2	3	0.64	0.91	1,650	-13	2,950	-51	5	4
3	2	70	76	2	2	0.64	0.83	1,650	-12	2,500	-15	4	3
4	2	70		1	2	0.64	0.79	950	+5	1,565	+69	3	3
5	2	70		1	2	0.54	0.93	125	-48	1,030	-33	3	2
6	2	70	75	1	1	0.54	0.66	17	-99	16	-86	1	1
7	2	70	74	1	1	0.58	0.70	113	-28	140	+14	2	2
8	2	69		1	1	0.55	0.67	81	+61	160	+30	3	3
9	2	70	74	1	1	0.55	0.64	265	-11	55	-43	3	2
10	2	68		1	2	0.62	0.89	135	+12	65	+30	3	2
11	2	68	80	2	3	0.86	1.04	215	+43	305	+228	3	3
12	2	70	75	2	2	0.65	1.00	45	+12	1,000	+56	4	3
13	2	70	76	2	2	0.66	0.96	360	-27	5,700	-47	3	3
14	2	70		1	2	0.66	1.04	200	+4	21	+575	3	3
15	2	72	74	1	2	0.56	1.05	145	None	15	+31	3	3
16	2	72	74	1	2	0.59	0.88	100	-51	20	+82	3	3
17	2	75	76	1	2	0.52	0.76	205	-56	11	+5	3	3
18	2	74		1	2	0.52	0.65	90	+10,329	2,150	-47	3	2
19	2	76	76	1	2	0.54	0.78	0.7	-73	1,370	+296	1	1
20	2	76		1	2	0.59	0.68	30	+133	56	-34	2	2
21	2	76	74	1	1	0.55	0.66	14	+1,233	1,090	+759	1	1
22	2	76	76	1	1	0.55	0.66	36	+275	32	+136	2	3
23	2	76	76	1	2	0.68	1.04	80	+59	540	+62	3	3
24	2	100	77	1	2	0.72	1.35	280	-7	525	+45	3	3
25	2	78	76	1	2	0.53	0.78	130	-43	19	+124	3	3
26	2	72	72	1	1	0.48	0.69	60	-58	500	+19	4	4
27	1	82	78	1	1	0.57	0.77	0.5	+1,790	18	-6	1	1
28	1	78	78	1	1	0.56	0.94	21	+156	73	+51	1	2
29	1	80	78	1	1	0.57	0.77	37	+74	340	+90	1	2
30	1	78	78	1	1	0.57	0.63	1,005	-47	645	+150	2	3
31	1	78	78	2	2	0.74	1.08	55	+473	550	+40	4	4
32	1	80	78	1	1	0.65	0.74	30	+1,350	425	+215	1	1
33	1	80	78	1	2	0.67	0.96	190	+73	755	+89	2	2
34	1	80	78	1	1	0.59	0.98	37	+22	680	-4	2	2
35	1	78	80	1	1	0.64	0.76	70	-49	145	+152	2	2
36	1	76	78	1	2	0.55	1.05	120	+105	365	+185	2	3
37	1	78	78	2	2	0.66	0.73	113	+24	167	+194	2	3
38	1	78	78	1	2	0.56	0.70	420	+15	90	+23	3	3
								73	+44	280	+214	3	3
								9	+1,111	85	+93	1	2

Following these preliminary studies a more detailed examination was made of 38 cans of cream and the results are presented in table 2. Changes in grade occurred frequently. At the beginning of the holding period 25 cans of cream were graded as first, 5 cans poor first, and 8 cans second. At the close of the holding period, 8 cans were considered first grade, 6 poor first, 22 second, and 2 third. In no case did the quality of the cream change more than one full grade during holding. The defects which developed to cause these changes in grade were of a considerable variety of types, no one defect or group of defects being characteristic. These data show that an appreciable increase in acidity occurred in all instances. The minimum increase was 0.06 per cent in a can of cream held one day, and the maximum increase was 0.63 per cent in a can of cream held two days. The average increase of the 38 cans of cream was 0.24 per cent.

The visual mold scores on the 38 lots of cream were found to remain unchanged in 21 instances, while increases in score occurred in 8 cans and decreases were noted in 9 cans. The maximum change in mold score was only one point. The number of cans of cream with a mold score of 1 decreased from 8 to 4, while the number of cans with a mold score of 2 increased from 9 to 14. Although five lots of cream were classed as doubtful or excessive (mold score 4 or 5) by the visual mold test when they were placed in holding, only two were so classified at the time the cream was shipped. Both lots of cream which were third grade at the end of the holding period improved one point in visual mold score during storage, the changes being from 5 to 4 and 4 to 3. Even in a few instances where mold mats appeared on the surface of the cream, the mold score on the mass of the cream was less at the end of the holding period than at the beginning. The results indicate that the mold content of cream originally containing only small amounts of mold hyphae tends to increase, while the mold content of cream containing large amounts of mold hyphae tends to decrease during holding.

The changes in visual mold score were paralleled reasonably well by the changes in mold plate count, although some discrepancies were encountered. The mold plate count of sample 7 increased from 165,000 to 265,000 per milliliter during holding, although the visual mold score dropped from 3 to 2. The increase in mold plate count from 14,000 to 180,000 per milliliter which occurred in sample 19 was in no way reflected in an increased visual mold score. This is especially significant since the cream dropped from first to second grade during the holding period. The visual mold scores on samples 10 and 24 were much higher than would be expected from the mold plate counts and really reflected the quality of the cream better than did the mold plate counts. Changes in yeast plate counts and mold plate counts were not parallel, either in direction or magnitude, in many cases, indicating that the numbers of each vary more or less independently.



Some of the microbiological data from table 2 are summarized in table 3. Among the samples obtained prior to holding, a tendency for a greater fraction of the samples grading number one to be placed in mold score classes 1 and 2 than in classes 4 and 5 was apparent, while more of the second grade samples were placed in the poor than in the good mold classes. After holding in the station, distribution on the basis of visual mold score was approximately the same for both first and second grade cream samples. The number of third grade samples was too small to permit the drawing of conclusions relative to them. The situation was similar with regard to distribution of samples according to mold or yeast plate counts, some relationship between count and grade being apparent before but not after holding. The data show that the tendency toward a relationship between organoleptic grade and visual mold score, mold plate count or yeast plate count which was observed before the cream was placed in holding was not evident at the end of the holding period. The microbiological changes observed failed to reflect the changes which had occurred in the organoleptically-determined quality of the cream during the holding period. These results indicate that the visual mold test in particular, but also the mold and yeast plate counts, are not as satisfactory for aiding in the grading of cream which has been held in the station as they are for the grading of producers' cream. This is a shortcoming of the visual mold test which apparently has not been recognized before.

The air space in the top of the cream cans was found to be fairly constant at 2 or 3 inches and differences apparently were not large enough to affect the results significantly.

#### DISCUSSION

Most investigators agree that the common dairy mold, *Oospora lactis*, which is usually the dominant mold in cream for buttermaking purposes, has little or no part in the development of the more serious flavor and aroma defects of cream. Most of the serious cream defects of microbial origin are the result of development of bacteria, although yeasts sometimes are involved. The value of the mold test lies in the fact that the conditions which permit molds to develop also will permit bacteria and yeasts to develop. Mold is easily detectable, while more complicated methods are necessary for approximate quantitative determination of bacteria and yeasts. If undesirable types of bacteria and yeasts are present they will produce defects under the same conditions that permit the molds to grow. Occasionally the molds may develop to a considerable extent while undesirable bacteria and yeasts do not develop. This may be because organisms of undesirable types were not present in the first place or because conditions were not satisfactory for development of organisms capable of causing pronounced defects. Such situations are potentially injurious to the quality of the cream, and the mold

test performs a definite service in indicating such possible sources of trouble, even though the situation may be under satisfactory control at the time the test is made.

Far more important from the standpoint of the evaluation of the mold test are the cases when poor cream may be missed when the mold test is employed as the only quality test. Molds require air for their development and most bacteria and yeasts grow satisfactorily in the absence of air. Under circumstances where the air supply is limited or lacking, mold development would be restricted markedly while development of bacteria and yeasts could continue unabated if other conditions were satisfactory. Under the anaerobic conditions in the depth of a large can of cream, mold could not develop and autolysis of the mold hyphae undoubtedly would occur. This would account for the reduction in mold count or visual mold observed during these investigations in some lots of cream originally high in mold content. This would also account for the greater tendency toward reduction of mold content after 2 days than was observed after one day. Under the conditions of this study, lack of air supply in the depths of the cans undoubtedly accounted for much of the discrepancy between mold content and quality of cream held in the cream station without adequate refrigeration.

Although the point is not important with regard to this study, the possible effect of temperature on comparative development of microorganisms should be mentioned. The rate of development of *O. lactis* is relatively rapid in the range from a little above 70° F. up to about 90° F. Above and below these temperature limits organisms responsible for more serious microbial defects of cream and butter could develop at a comparatively much more rapid rate than would the mold. This would seriously interfere with the correlation between mold content and quality of cream or butter.

The results of this study indicate that the mold test must be used with discretion, and in conjunction with other tests for the proper evaluation of cream quality. Unquestionably the test has considerable value for the grading of cream as it comes from the farm. The test has only limited applicability for grading cream which has been held for even twenty-four hours without adequate refrigeration in cream stations, because conditions in such stations usually are not favorable for appreciable mold development but definitely are favorable for the development of bacteria and sometimes yeasts.

#### SUMMARY AND CONCLUSIONS

The effect of holding cream in the buying station on the mold content and the quality of cream was studied. The cream was held in 10-gallon cans for one or two days at slightly below atmospheric temperature. A total of 113 lots of cream was examined.

The results indicate that the visual mold test used as an index of the

quality of cream for buttermaking does not reflect the changes which occur during holding in the cream station. The quality of cream as indicated by grade and acidity was found to decrease rapidly during holding in the cream station, while the visual mold score frequently did not reflect the deterioration in quality which occurred.

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## DEVICES FOR MEASURING PHYSICAL PROPERTIES OF CHEESE

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Each variety of cheese has certain typical physical characteristics. For example, Swiss cheese should have a pliable and elastic curd, sufficiently close-grained to hold the gas formed during ripening and capable of stretching so that eyes are formed with smooth, glistening surfaces. Failure to obtain this texture is indicated by irregular eyes with rough or "nut like" surfaces, an obviously tough curd, or a weak curd which cracks when the eyes are formed. These characteristics are easily recognized and roughly evaluated by the experienced cheesemaker or dealer, but in experimental work it is desirable to have a means of measurement of these properties and to be able to express this measure in mathematical terms.

For determining the firmness of curd at the time of cutting, Scott Blair (4) devised an instrument to measure the depression of the curd under a 300-gram weight held in a light tray and the recovery of the curd when the weight is removed. Firmness of curd at various later stages in the making process was determined by Vas (7) by means of a "coagulometer," which measures the distance through which a flat plate sinks into a 20-gram sample of curd, contained in a perforated chamber, in 5 minutes under a pressure of 500 grams. A device for measuring the consistency in terms of superficial density in Cheddar and other varieties during the heating process has been used by Scott Blair and Coppen (5) as a means of establishing uniformity in "pitching" time (stage at which curd is allowed to settle).

For measuring physical properties of cheese, a penetrometer which indicates firmness in terms of the distance that a plunger sinks into a sample under a definite pressure has been devised by Koestler (2) for Emmentaler and similar varieties, and one which shows the weight in grams required to push a cone-tipped shaft into a sample has been devised by Roundy and Price (3) for measuring the consistency or "body" of cream cheese. Devices for measuring the amount of deformation occurring in a small block of cheese when subjected to a definite pressure, and the amount of elastic recovery when the pressure is removed, have been used by Koestler (2) and also by Davis (1), who described the basic, rheological principles concerned in the measurement of body and texture in cheese, butter, etc. A device and technique have been described by Scott Blair and Coppen (6) for use with cured cheese to measure the deformation produced by pressing a spherical skewer 1.5 inches in diameter with a weight of 36 pounds against the top surface of the cheese—a method designed to imitate the pressure of the

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thumb on the cheese as a measure of cheese "body." A device has been described by Koestler (2) for measuring the amount of elastic stretch occurring in a rectangular, bar-shaped sample when the ends are pulled in opposite directions and also the amount of subsequent recovery when the stretching force is removed.

It has been found desirable in our work to establish a definite standard of firmness of curd at the time of cutting, by a method which would be more sensitive than the one mentioned above, and whose results would be less subject to variations caused by differences in the size of the container (beaker, vat or kettle) and less masked by the buoyancy of the liquid. Such type of measurement is obtained with the Hill curd tester (American Curd-O-Meter), but it is not suitable for use on a vat of milk and not sufficiently sensitive to measure the small differences existing in milks in the early stages of curdling. In measuring plasticity of cheese in terms of firmness, difficulty was encountered in securing a method applicable to samples varying greatly in consistency, and to samples of different varieties. In measuring elasticity it was difficult to prevent slippage when attempts were made to fasten each end of a bar-shaped sample in holding devices, and samples both sufficiently large and free of "weak" areas were difficult to secure from Swiss cheese which contained small eyes or cracks. The three instruments described below were designed in an attempt to overcome the difficulties mentioned.

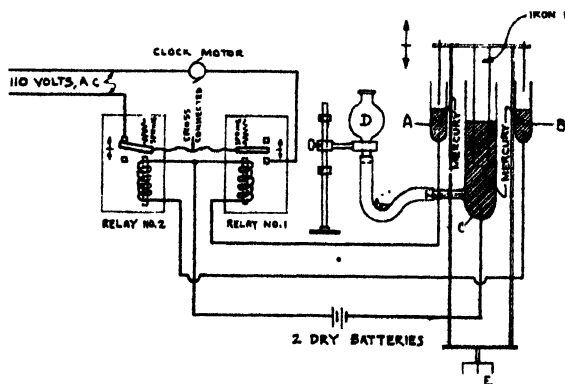


Fig. 1. Instrument for measuring the firmness of curd at cutting time.

#### CURD TENSION METER FOR VAT OR KETTLE MILK

The instrument is a modification of the Hill meter and includes the standard knife or cutting "head" of this instrument. Firmness of rennet-coagulated curd is determined by measuring automatically the time required for the cutter, when actuated by a controlled force, to move a definite distance through the coagulating milk in either a beaker or a vat or kettle. The construction is shown diagrammatically in figure 1. An iron rod weigh-

ing about 200 grams floats freely in mercury in a glass cylinder and supports a yoke which carries the cutter (E) below the cylinder. The level of the mercury in the cylinder, and consequently the position of the float, is regulated by a leveling bulb (D) connected to the cylinder through a capillary. The leveling bulb is supported on a rod with stops which limit its vertical movement. The measuring device is mounted, with a ball and socket joint to permit leveling, on a rod fastened to a suitable base so that it can be supported over a cheese kettle and so adjusted that the cutter, in its upper position, is just clear of the surface of the milk.

When the leveling bulb is at its upper position, the mercury flows into the cylinder and raises the float and the cutter. When it is dropped to its lower position, the mercury runs slowly from the cylinder into the leveling bulb and the cutter moves downward through the curd until the float is again in equilibrium with the mercury. The internal friction is negligible and, since the pressure on the cutter caused by the falling of the mercury level is under positive control, the rate of movement of the float and cutter is a direct function of the weight applied and an inverse function of the resistance offered to the cutter by the curd.

The time required for the cutter to move through a certain distance is measured automatically through contact wires on the yoke of the float so adjusted that one makes contact in a mercury cup (A) at the beginning of the movement and the other makes contact in a second mercury cup (B) and closes another circuit at any predetermined point in the fall of the float. These mercury cups are connected by wires with two relays, of which No. 1 is normally open and No. 2 normally closed. The relays are connected and mounted in a cabinet with an electric timer which has a dial graduated to  $\frac{1}{2}$  second and the graduations so spaced that  $\frac{1}{10}$  second may be estimated accurately.

The mercury level in cup A is so adjusted that the contact wire is 2 or 3 mm. above the mercury when the leveling bulb is in its upper position. When the bulb is lowered to the bottom stop, the mercury flows slowly through the capillary and the downward movement of the float closes the circuit through cup A, thus activating relay No. 1 and starting the clock. When the contact is made in cup B, relay No. 2 is opened and the clock is stopped.

The sensitivity of the instrument is decreased but a firmer curd may be measured if the bottom stop is lowered. This has the effect of putting a larger proportion of the weight of the float on the cutter and forcing it through a curd in which it would otherwise stall. For measuring the firmness of the curd at cutting we have found satisfactory a movement of the float of  $1\frac{1}{2}$  inches and a rate of flow through the capillary that will require 16 to 18 seconds between the time the contacts are made in A and in B. With this adjustment incipient coagulation will be indicated by a more or

less marked increase in the time required for the cutter to pass through the sample. The measurements are relative and may be expressed as the number obtained by dividing the time required for the cutter to move a certain distance in milk or curd (A) by the time required to move the same distance in air (B) ( $\frac{A}{B}$  = firmness of curd).

The readings obtained on a kettle of typical Swiss cheese milk are shown in figure 2.

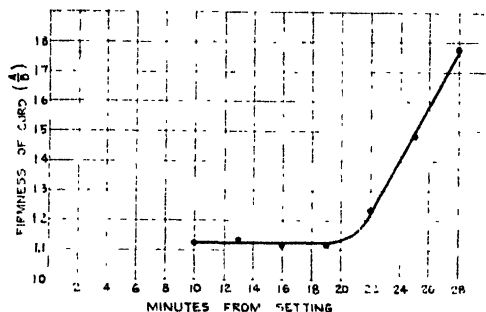


FIG. 2. Increasing firmness of curd under the action of rennet as measured in a typical kettle of Swiss cheese milk.

#### ELASTOMETER FOR CHEESE

Of the various methods by which elasticity in terms of ability to stretch may be measured, the most direct is to measure the elongation of a beam of curd under a definite pull. An instrument was devised to do this, and results obtained with it were reasonably consistent and, in most cases, correlated satisfactorily with other characteristics of the cheese. It was necessary to cut from a plug of cheese a dumbbell-shaped bar of curd free from cracks or other defects, which could be held without slippage or danger of crushing. In overset or otherwise defective cheese it was difficult, and frequently impossible, to secure samples which would meet these requirements.

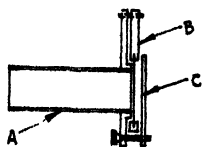


FIG. 3. Device for cutting disks of uniform thickness from a plug of cheese.

A second instrument was constructed which required a disk of curd 18 mm. in diameter and of uniform thickness. Disks of suitable dimensions were secured by cutting a plug of the required diameter with a cork borer and cutting disks from it with a special cutter (fig. 3) consisting of a tube (A) through which the plug of cheese is pushed against an adjustable stop (C), and a thin, sharp blade (B) which swings on a pivot and bears against

the end of the tube. To measure elasticity, the disk-shaped sample (G, fig. 4) is held in place in the top of a metal chamber (A) by a collar secured by two set screws, preventing leakage of air. The chamber, which is supported on a stand so that its position is adjustable, is then raised so that the surface of the disk touches the foot of a thickness gauge (B) with the indicator at zero. The weight of the moving parts of the gauge is balanced on a pulley with a light cord and a counterweight. Air is admitted into the chamber by opening a stopcock (D) and the amount of deformation of the sample is read on the gauge, which is graduated to 0.001 inch. Since the bulging of

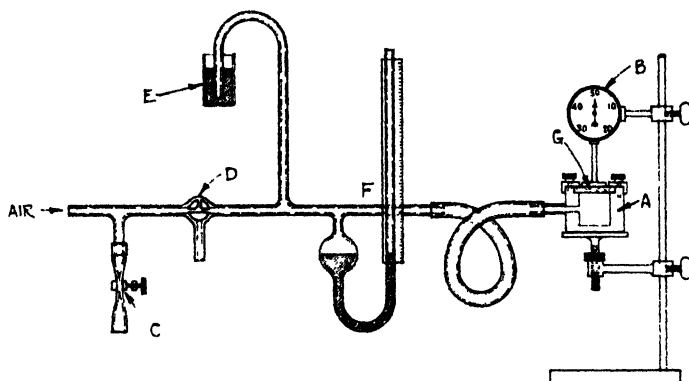


FIG. 4. Instrument for measuring the elasticity of Swiss cheese.

the sample usually continues until it is ruptured, it is necessary to read the gauge at the end of a definite time from the instant at which air has been admitted. Uniform pressure is obtained through a glass connection provided with an adjustable leak (C) which holds the pressure within safe limits, a mercury leak (E) by means of which the pressure in the chamber (A) may be maintained at any desired point, and a manometer (F). The

TABLE 1

*Variations in physical properties of Swiss cheese made from milks differently treated*

Treatment of milk	Plasticity of cheese		Elasticity of cheese
	Green	Cured	Cured
	pounds	pounds	0.001 inch
Control		26.5	147
1% concentrated whey added		11.3	63
Control	26.2	17.5	65
25% whey added	20.0	12.2	157
Control	26.4	17.0	95
25% whey added	25.0	11.6	120
Control		23.0	31
<i>L. casei</i> culture added		15.8	166

measurements are made in a constant-temperature room in which the cheese samples have been held long enough to be sure that their temperature will have reached that of the room.

Examples of results obtained with typical experimental cheese are shown in table 1. These readings were obtained at a temperature of 20° C. on disks 2 mm. thick exposed to a pressure of 2.5 cm. of mercury for 1 minute.

#### PLASTOMETER FOR CHEESE

One of the most significant physical characteristics of Swiss cheese is the property usually called toughness, but which we refer to as relative plastic-

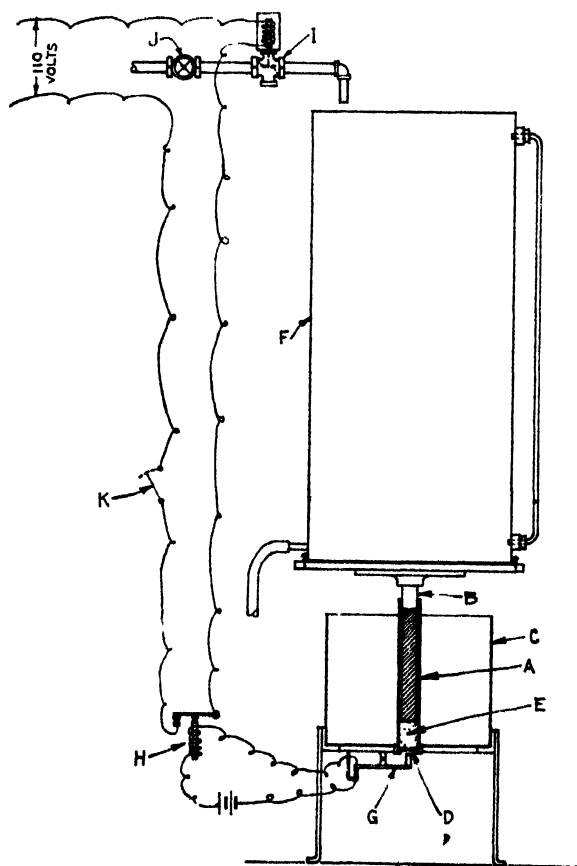


FIG. 5. Plastometer for measuring the plasticity or toughness of Swiss and other varieties of cheese.

ity. It may be evaluated by a penetrometer test, and we have used such an instrument to some extent. However, we have found more satisfactory a special instrument by which small differences in toughness may be readily

detected, regardless of the presence of small gas holes or cracks, and expressed in mathematical terms.

The essential parts of this apparatus, which is shown in figure 5, are a cylinder (A) in which a plug of cheese is subjected to pressure sufficient to cause it to flow, a detachable base plate (D) containing an orifice through which the cheese flows, and a piston or plunger (B) to supply pressure on the sample. In the method as first used, the cheese was subjected to a definite pressure for a definite time and the amount of cheese which was forced through the opening was determined by weighing it. This method did not permit accurate measurements on samples so soft that the entire plug was forced through the opening, or on those so tough that no cheese went through. The procedure was altered to measure the pressure required to produce the beginning of flow through the orifice, and the equipment can be used, therefore, at any stage of ripening and for all varieties of cheese. As used in our work the cylinder (A) is  $\frac{1}{2}$  inch in diameter and  $7\frac{1}{2}$  inches long. The base plate (D) is 0.20 inch thick and the orifice in it is 0.063 inch in diameter. The piston (B), which is machined to a good fit, moves freely and supports a circular platform 9 inches in diameter. Pressure is supplied by water running into a can (F) on the platform, and the can has a gauge extending its entire length and calibrated to show directly in pounds the total pressure on the sample. For extremely soft varieties in which the pressure required is less than 5 pounds, the weight of the upper, movable parts (can and plunger) can be balanced by suspending them on a pulley with a cord and a counter-weight.

The cylinder is enclosed in a water bath (C) to insure a uniform temperature of the cheese sample (E) previous to and at the moment of testing. Additional samples to be tested are placed in glass tubes in the same water bath. In operating the equipment, a 9-gram plug of cheese, taken with a trier, is placed in the cylinder and its temperature is allowed to reach that of the bath ( $26-26.5^{\circ}$  C.). The piston, lubricated lightly with petrolatum, is inserted above the sample with the instrument set so that the platform is level. Pressure is applied by closing a switch (K) in the 110-volt circuit, thus opening a magnetic valve (1) and causing water to flow into the can at a constant rate of 8 pounds per minute. The rate of flow is regulated by a valve (J).

The pressure increases gradually and eventually becomes sufficient to press cheese through the orifice to push down a switch-arm (G), which is held 0.035 inch below the lower end of the orifice by a spring, permitting movement under a pressure of 1 gram. This opens the low-voltage circuit activating a relay (H) which in turn closes the magnetic valve, stopping the flow of water into the can. The pressure is read on the gauge.

Typical measurements of relative plasticity are shown, with those of elasticity, in table 1. A high plasticity reading indicates relatively great

toughness, while a high elasticity reading shows a high degree of ability to stretch or bend. It will be noted that these two properties are not necessarily correlated but that each is affected very definitely by differences in the making process.

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# THE ADVANTAGE OF GRINDING ATLAS SORGHUM GRAIN FOR DAIRY COWS\*

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Due to the large increase in acreage of sorghums in Kansas, much interest has arisen among dairy farmers in the use of sorghum grains as a concentrate feed. Atlas sorgo (16) is a sweet sorghum used extensively as a forage crop for silage in Kansas and at the stage of maturity usually cut for silage, it may yield considerable quantities of mature grain (19, 20). It is also grown as a grain crop in certain areas of the state.

Any grain in the dairy cow ration which escapes mastication will pass through the digestive tract as whole grain. Grinding grain for dairy cows is a common practice, the degree of fineness of the grinding depending to some extent upon the kind of grain fed.

Sorghum grain is small and hard and is more apt than some other grains to pass through the cow. The protective hull of the seed must at least be cracked in order to permit the digestive juices to act most effectively upon the nutrients in the grain.

Observation on the whole sorgo grain which passes through the cow, whether fed as silage or as a concentrate, has caused farmers to inquire about the losses involved, the desirability of grinding the grain, and how fine to grind it.

## REVIEW OF LITERATURE

Several investigators (5, 6, 7, 10, 12, 14, 15, 17, 18, 19, 20, 21) have reported on the advantages of grinding various grains, such as corn, oats, and barley, as a feed for dairy cattle. As early as 1902, Otis (13) commented on the large amount of kafir corn that passed through six-months-old experimental beef calves.

The value of ground sorghum grains as a feed for dairy cattle has been established (3, 4, 6, 7). Cave and Fitch (3) were the first to present data on the waste resulting from the feeding of sorghum crops. They reported as high as 90 per cent of the seed in sumac silage passed through the cow undigested. When they fed kafir silage they found about 30 per cent passed through. LaMaster and Morrow (11) found that about 38 per cent of the seed in sweet sorghum silage passed through dairy cows unmasticated.

Fitch and Wolberg (8) reported that the seeds in Kansas Orange sorgo and Atlas sorgo silages were utilized slightly better by dairy cows than were the seeds from these plants when fed as whole grain. When Kansas Orange

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silage was fed with alfalfa hay and a grain mixture an average of 43 per cent of the seed in the silage was recovered in the feces; while an average of 36 per cent of the seed in Atlas sorgo silage was recovered. When fed as the only concentrate with alfalfa hay, an average of 62 per cent of the Kansas Orange seed was recovered, and 51 per cent of the Atlas sorgo seed. When Atlas sorgo silage was fed as the only feed the recovery averaged 30 per cent. The effect of the other feeds fed is of doubtful significance because the difference between the average recovery of Atlas sorgo seed from silage (36 as compared with 30) was not so great as the differences between individual cows in each trial.

Darnell and Copeland (6, 7) reported an average grain recovery in the feces of dairy cows of 67.8 per cent when whole milo was fed, 30.5 per cent when whole corn was fed, and no satisfactory recovery from whole oats. They concluded that, "... size of the grain is a factor in the amount of whole grain masticated." No other published data were found pertaining to measured losses of sorghum grain when fed to livestock.

Hogs usually chew their grain more thoroughly than cattle, but even with hogs, Hale (9) found that when they were hand-fed on whole kafir, 10 per cent of the feed was recovered as whole grain in the feces. When the hogs were self-fed, 2 per cent of the seed passed through unmasticated.

Although, as mentioned above, several investigators have reported on the feeding value of ground sorghum grains as a feed for dairy cattle, while others have presented data on the loss of whole grains in feces, no publications have come to the attention of the writers in which data on the savings due to grinding were presented.

#### EXPERIMENTAL PROCEDURE

The effect of fineness of grinding on the per cent of sorgo grain apparently passing through the cow was determined by feeding Atlas sorgo grain as whole grain, coarsely ground, and finely ground.

Two dry cows were selected, one Holstein and one Jersey, which had previously been fed on the normal herd ration. They were fed through three ten-day periods on a ration of alfalfa hay *ad libitum*, and sorgo grain at the rate of about ten pounds per head daily. During the first period whole grain was fed; in the second, coarse ground or cracked grain; and in the third, finely ground grain.

All the feces voided during the last three days of each ten-day period were collected. Grain was recovered from the feces by working and washing in a deep barrel and allowing the grain to settle, then decanting the liquid refuse. This washing and decanting process was repeated until the grain became fairly clean. It was then necessary to air-dry the grain and again repeat the washing and decanting in order to remove all of the foreign material. After the final washing the grain was air-dried and weighed.

Undoubtedly some finer particles of the grain were lost in the washing and decanting process. The amount of grain found in the feces represents a minimum amount that passed through the cow.

In Kansas, many farmers feed cows almost exclusively on the sorghum plant, using the fodder as dry roughage, either with or without sorgho silage, and the sorgho grain as the major or exclusive grain feed. Field observations and experiments in progress indicate that cows on such rations become unthrifty; and the question arose whether cows on such rations would utilize the sorgho grains to more or less extent than cows on better balanced rations. Three groups of cows which had been fed fixed rations for more than a year were available. Group I had been fed Atlas sorgho fodder, Atlas sorgho silage, and a concentrate of Atlas sorgho grain only. Group II was fed the same, except enough cottonseed meal and bone meal were fed to meet the protein and mineral requirements of Morrison's standards. Group III had been fed alfalfa hay, sorgho silage, and a grain mixture consisting of 400 pounds of ground Atlas sorgho grain, 200 pounds of wheat bran, and 50 pounds of cottonseed meal.

Cows in groups I and II were in an extremely thin condition, having lost from one-fourth to one-third of their body weight since being put on the experimental rations. The cows in group III were in good condition, apparently normal.

Since it seemed, from the appearance of the feces, that the cows in groups I and II were wasting more of their grain than normal cows, it was decided to study two cows from each of the three groups along with the previously mentioned two cows from the college herd, used as a check.

Because it was not desirable to make any changes that might upset the results of the other experiment, the cows selected from groups I, II, and III were omitted from the first feeding period during which whole grain was fed; the only change made in the rations fed these cows during the second and third feeding periods was to use grain of the same grind that was being fed to the two dry cows.

The amount of grain fed the cows in groups I, II, and III varied considerably due to differences in their feed requirements, while the check cows were fed all they would readily consume.

As a check on the utilization of sorgho grain consumed in silage as compared with grain fed as a concentrate, a dry Jersey cow was fed exclusively on Atlas sorgho silage at the rate of 30 pounds daily. The percentage of grain in the silage was estimated by hand picking the grain from representative samples.

Both the Atlas sorgho grain and the Atlas sorgho silage used in these experiments were grown in the vicinity of Manhattan, during the crop year of 1939. The grain was rather typical, although possibly the seeds were not so large and plump as in some years. The silage was less mature and the

TABLE I

*Utilization of Atlas sorgo seed when fed to dairy cows as whole seed, coarsely ground, finely ground; and as grain feed compared with grain in silage*

Groups	Check (dry cows)												I					
	152 Holstein				318A Jersey				Average				374 Jersey		E44 Holstein		Average	
	Whole	Coarse grind	Fine grind	Whole	Coarse grind	Fine grind	Whole	Coarse grind	Fine grind	Whole	Coarse grind	Fine grind	Coarse grind	Fine grind	Coarse grind	Fine grind		
Form of sorgo grain																		
Trial I																		
Sorgo grain consumed daily (lbs.)	8.6	10.0	9.1	7.7	10.0	7.3	8.1	10.0	8.2	4.0	4.0	2.0	2.0	2.0	3.0	3.0		
Sorgo silage consumed daily (lbs.)										14.0	13.8	20.0	20.0	20.0	17.0	16.9		
Grain in silage consumed daily* (lbs.)										0.18	0.18	0.26	0.26	0.26	0.22	0.22		
Total grain recovered in feces (lbs.)	3.30	0.37	0.10	3.50	0.60	0.13	3.40	0.48	0.12	1.00	0.33	0.43	0.43	0.40	0.71	0.36		
Recovered grain originating from silage* (lbs.)										0.02	0.02	0.03	0.03	0.03	0.02	0.02		
Total grain recovered, minus grain from silage (lbs.)										0.98	0.31	0.40	0.40	0.37	0.69	0.34		
Concentrate grain recovered (%)	38.4	3.7	1.1	45.5	6.0	1.8	42.0	4.8	1.5	24.5	7.8	20.0	18.5	23.0	11.3	11.3		
Trial II																		
Sorgo grain consumed daily (lbs.)										4.6	4.6	5.4	5.4	5.4	5.0	5.0		
Grain recovered in feces (lbs.)										1.0	4.5	1.3	0.7	1.15	0.57	0.57		
Grain recovered (%)										21.7	9.8	24.1	13.0	23.0	11.4	11.4		

\* Grain in silage was determined by hand picking representative sample (1.3%); grain recovered from silage was computed by using the figure (10.7%) obtained cow fed silage only.

TABLE 1—(Continued)

Utilization of *Atlas* sorgo seed when fed to dairy cows as whole seed, coarsely ground, finely ground; and as grain feed compared with grain in silage

Groups	II						III						Silage fed cow
	492 Guernsey		273 Ayrshire		Average		381 Jersey		495 Guernsey		Average		
	Coarse grind	Fine grind	Coarse grind	Fine grind	Coarse grind	Fine grind	Coarse grind	Fine grind	Coarse grind	Fine grind	Coarse grind	Fine grind	
Ear tag number and breed													
Form of sorgo grain													
Trial I													
Sorgo grain consumed daily (lbs.)	1.2	1.2	4.1	4.1	2.6	2.6	6.0	6.0	6.0	5.0	6.0	5.5	30.0
Sorgo silage consumed daily (lbs.)	20.0	20.0	21.2	16.8	20.6	18.4	20.0	17.7	22.0	18.4	21.0	18.0	
Grain in silage consumed daily* (lbs.)	0.26	0.26	0.28	0.22	0.27	0.24	0.26	0.23	0.29	0.24	0.27	0.23	0.39
Total grain recovered in feces (lbs.)	0.30	0.13	0.60	0.27	0.45	0.20	0.37	0.17	0.16	0.16	0.27	0.17	0.04
Recovered grain originating from silage* (lbs.)	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.02	0.03	0.02	0.03	0.02	0.04
Total grain recovered, minus grain silage (lbs.)	0.27	0.10	0.57	0.25	0.42	0.17	0.34	0.15	0.13	0.14	0.24	0.15	
Grain recovered (%)	22.5	8.3	14.0	6.1	16.2	6.5	5.7	2.5	2.2	2.8	4.0	2.7	
Trial II													
Silage	3.7	3.7	3.7	3.7	3.7	3.7	6.2	6.8	7.2	7.2	6.7	7.0	
	0.76	0.42	0.7	0.38	0.73	0.4	0.14	0.1	0.2	0.07	0.17	0.08	
	18.9	10.3	18.9	10.3	19.7	10.8	2.3	1.5	2.8	1.0	2.5	1.1	

and picking representative sample (1.3%); grain recovered from silage was computed by using the figure (10.7%) obtained from

heads contained much less seed than usual due to deficient rainfall during the growing season.

#### RESULTS

The Atlas sorgo grain fed to the two dry cows from the college herd, using as a check, resulted in an average of recovery in the feces of 42.0 per cent of the grain when fed whole, 4.8 per cent when the grain was coarsely ground, and 1.5 per cent when finely ground (table 1).

The grain recovered in the feces from the cows in group III, which had been receiving a rather typically normal ration, compared quite closely with the check group in spite of the fact that these cows received silage while the check cows did not. The recovery on coarse ground grain averaged 4.0 per cent, and 2.7 per cent on fine ground. In group II the average recovery for coarse ground grain was much higher, being 16.2 per cent for coarse ground grain, and 6.5 per cent for fine ground. Group I was even less efficient than group II, the average recovery of coarse ground grain being 23.0 per cent, and 11.3 per cent for fine ground. Groups I, II, and III ranked in grain utilization in the same order of their general appearance as to thrift.

After the first feeding trial of three periods was completed, it appeared that the grain in the silage fed to the cows in groups I, II, and III might prevent direct comparison of the results with those obtained from the check cows fed no silage. Therefore, the trial was repeated using the same two cows in groups I, II, and III fed the same rations as before, except that silage was removed from the ration. The Atlas sorgo grain was ground to the same fineness modulus as in the first trial.

Comparison of groups I, II, and III with the check cows was complicated by the fact that the check cows received no silage while the other three groups were fed sorgo silage. It was found that the grain represented only 1.3 per cent of the weight of the silage as fed. When a cow was fed exclusively on this silage at the rate of 30 pounds daily, 10.7 per cent of the grain was recovered in the feces. This figure was used in computing the amount of grain represented by silage which was recovered in the feces of groups I, II, and III in trial I. Due to the small amount of grain in the silage, the cows in these three groups did not receive enough grain from that source to permit comparison with the check group of cows fed no silage.

In the second trial, the quantity of sorgo grain fed was increased. In an average of 2.5 per cent of the grain was recovered in the feces when it was coarse ground, and 1.1 per cent when finely ground. In an average of 19.7 per cent of the coarse ground grain was recovered in the feces, and 1.1 per cent of the fine ground. The recovery of grain in group I was 23.0 per cent for coarse, and 11.4 per cent for fine. The three groups ranked in the same order as before and the results compared quite close with the previous trial, when silage was fed. In

fact, the average of the two trials checked more closely than did the individual cows within a group. The results, however, as shown by the data on individual cows were quite consistent.

#### DISCUSSION

When whole sorgo grain is fed as a concentrate, some grain cracked by mastication might also be expected to pass through the digestive tract, since it was shown in these trials that some ground grain passed through. The grain recovered from the feces, however, did not contain any more cracked grain than did the original whole grain fed. Several investigators (1, 7, 8, 11, 14, 17, 21) have reported that no appreciable digestion of whole grain occurs when passing through the cow, as measured by chemical analyses. The results on the recovery of ground grain might be less accurate than for whole grain. Wilbur (21) reported that starch analyses of feces were closely related to the amounts of grain passing through the cows undigested when whole corn and oats were fed. Thallman and Cathcart (17) found that when corn processed in various ways was fed to beef cattle, there was a close relationship between the results measured by grain passing through and by digestion trials. It is safe to conclude, however, that at least the amount reported passed through the cow; and since such a large percentage of whole grain was voided the waste is great; while the loss of ground grain is relatively small, even allowing for error.

The loss of 42 per cent of the grain in the feces when the grain was fed whole indicated the necessity for grinding. The results are in general harmony with the findings of Fitch and Wolberg (8) who reported a waste of 51 per cent for Atlas sorgo fed as a concentrate, and with other investigators (1, 6, 7, 8) using different varieties of sorghums. Differences can be attributed to differences in varieties, maturity of seed, and growing conditions.

Medium ground grain has been recommended for dairy cattle (2, 15, 21). Coarse ground sorgo grain was compared with finely ground grain in these trials in order to make any differences more significant. Also, sorgo seed is so small that coarse grinding, if properly done, would make it typical of medium grinding of some other grains. The finely ground grain represented a fineness modulus of 2.44, which was as fine as it was possible to grind it. The coarse ground grain was ground to a fineness modulus of 3.65. Whole Atlas sorgo grain represents a fineness modulus of 4.0. A hammer mill was used for the fine grind and a burr mill for the coarse grind. The energy consumption per hundred pounds of grain was 0.158 K.W.H. for the coarse grind, and 0.502 K.W.H. for the fine grind.

<sup>1</sup> Grinding of grain to specific fineness modulus was done by the Department of Agricultural Engineering, Kansas State College. Data for grinding were also furnished by him.

increased cost of fine grinding is typical of reports on the grinding of other grains (2, 15, 21).

Compared with an average waste of 42 per cent when the grain was fed whole, grinding of the grain resulted in a great saving. An average recovery of only 4.8 per cent of the coarsely ground grain from the feces of the check group, and 4.0 per cent for group III when fed silage and 2.5 when silage was omitted; compared with an average recovery of 1.5, 2.7, and 1.1 respectively, for the finely ground grain indicates that coarse grinding is most satisfactory considering cost of grinding, saving of waste, and consistency of feed.

In the group of cows (I) which had been fed for more than a year on an unbalanced ration derived exclusively from the sorgo plant, the grain waste averaged more than five times as much as in the cows fed more normal rations. Group II, which received the same rations as group I, except for the addition of cottonseed meal and bone meal, utilized their grain better but were much more wasteful than the cows fed more normal rations. Why the cows in groups I and II were unable to utilize their grain as well as the cows fed good rations is not known; but this fact is in agreement with the production and appearance of these cows compared with normal herd cows. It also emphasizes the poor economy of such rations.

It is interesting to note that a considerable increase in grain consumption for groups I, II, and III during the second trial, when silage was omitted, did not change significantly the average percentage loss of grain in the feces as compared with the first trial.

The fact that an average of only 10.7 per cent of the grain in the silage was recovered from the feces might lead to the conclusion that the waste of grain in silage is not excessive. Such is not the case, however, as the silage used in this trial was immature, indicated by the fact that grain represented only 1.3 per cent of the silage by weight as fed. Fitch and Wolberg (8) found that 36 per cent of the seed in Atlas sorgo silage, and 43 per cent of the seed in Kansas Orange sorgo silage passed through the digestive tract of the cow and were recovered as whole seed from the feces. Cave and Fitch (3) had previously reported as high as 90 per cent of the sorgo seed in silage had passed through the cow undigested. The waste of grain in silage may be so great in good crop years that Weber (19, 20) has investigated the merit in separating the heads separately and blowing them into the silo with the cut sorgo. When fed to beef cattle, the silage containing ground heads resulted in a net gain of 19 per cent one year, and 12 per cent the next. In good growing conditions, and crop variety would influence the value of sorgo silage is fed.

#### SUMMARY AND CONCLUSIONS

In this experiment 12 dairy cows were conducted to determine the value of

grinding of Atlas sorgo grain as measured by the amount of grain recovered in the feces. Two dry cows (check group) were fed during three ten-day periods on alfalfa hay plus a concentrate of: 1. Whole Atlas sorgo grain, 2. Coarsely ground sorgo grain, and 3. Finely ground grain. Three other groups (I, II, III) were fed the Atlas sorgo grain, ground to the same fineness modulus, both with and without silage. Group I had been receiving for more than a year an experimental ration restricted to the Atlas sorgo plant—sorgo fodder, sorgo silage, and sorgo grain. Group II had been fed the same except cottonseed meal and bone meal were included. Group III received alfalfa hay, sorgo silage, and a grain mixture of sorgo grain, wheat bran and cottonseed meal. Another cow from the college herd was fed exclusively on silage to determine the amount of grain in silage passing through the cow undigested.

Feeding whole grain resulted in excessive waste while coarse grinding was more satisfactory than fine grinding, considering cost of grinding and consistency of feed. In the check group the recovery of grain in the feces averaged 42 per cent for whole grain, 4.8 per cent for coarsely ground, and 1.5 for finely ground. The grain recovered from cows in Group III averaged 4.0 per cent for coarsely ground and 2.7 per cent for finely ground, when silage was fed; and 2.5 and 1.1 per cent, respectively, when silage was omitted.

Cows receiving non-typical experimental rations did not utilize their grain feed efficiently. In group II, when silage was fed, the recovery of grain in the feces averaged 16.2 per cent for coarse grind, and 6.5 for fine grind; while when silage was omitted, 19.7 and 10.8 per cent, respectively, were recovered. Results on group I showed an average recovery of 23.0 per cent for coarse grind, and 11.3 per cent for fine, when silage was fed. When silage was omitted, the recovery averaged 23.0 and 11.4 per cent, respectively.

Increasing daily grain intake (groups I, II, III) when silage was omitted did not significantly change the percentage of grain recovered from the feces.

The silage fed was immature, the grain content as fed being 1.3 per cent. The recovery of grain from the feces of a cow fed exclusively on this silage averaged 10.7 per cent.

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## THE SIGNIFICANCE OF TANNIC SUBSTANCES AND THEOBROMINE IN CHOCOLATE MILK\*

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When mineralized chocolate milk contained 2.5 per cent by weight of cocoa powder and was the sole source of food, the average daily consumption of cocoa per rat was approximately 1 gram or 10 grams per kilogram body weight (5). This amount of cocoa was toxic for rats and the toxicity increased with an increase in cocoa intake. Most of the chocolate milk sold commercially contains less than 2.5 per cent cocoa, but as the beverage is prepared in the home it may contain as much as 3 per cent. There are other chocolate or cocoa-milk products which have a cocoa concentration greater than 2.5 per cent. For example, chocolate ice cream may contain 3.5 per cent cocoa, and sweet milk chocolate candy as much as 12 per cent cocoa. On the basis of these percentages the calculated cocoa intake per day for children may be 30 grams or 1 gram per kilogram body weight, when the recommended quart of milk per day is given as chocolate milk and the diet includes other cocoa-containing foods. Thus, the approximate daily cocoa intake per child on the basis of weight is only one-tenth that which was toxic for rats.

To the writer's knowledge no experimental proof is available which shows definitely what constituents of cocoa are chiefly responsible for its toxicity to rats. Theobromine is usually suspected as being the most harmful constituent of cocoa, although it is present in only small quantities (0.7 to 2.7 per cent). However, much that has been written about the harmful effects of this alkaloid is full of contradictions.

Another constituent of cocoa, present in much greater amounts than theobromine, is the group of tannic substances. The chemical properties of these substances and the amount present leads one to suspect that they may be chiefly responsible for the toxicity of cocoa. Kuzmeski and Mueller (3) analyzed eighteen samples of commercial cocoa powder for cacao-red, and found values ranging from 2.62 to 15.59 per cent. According to Knapp (2) the tannic substances in cacao beans after fermentation and drying are: red-brown products, dark-brown products, tannin, and cacao-purple, which is identical to cacao-red. Since comparatively little is known about the group of tannins in cocoa they will be referred to throughout this paper simply as tannin-like or tannic substances.

Since cocoa and chocolate are being consumed in increasing amounts, practical methods by which the toxicity may be overcome seemed desirable. To obtain such information this study was undertaken.

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## GENERAL PLAN OF STUDY

To determine the physiological effects, pure crystalline tannic acid and pure theobromine were added to whole milk powder and fed to albino rats. Other groups of rats were fed whole milk powder plus cocoa powder which varied widely in tannic substances content, but were similar in most other respects. Also, a concentrated extract of cocoa powder, which was practically free of tannic substances and theobromine was added to fluid whole milk and fed to rats. The criterion for determining relative toxicity was growth rate, general appearance and well-being of the animals. Hemoglobin content of blood was also determined.

## EXPERIMENTAL

*Feeding crystalline tannic acid and theobromine.* In this study twenty-four young rats, twelve males and twelve females, were divided into eight groups. Each group of three animals consisted of litter mates of the same sex and as nearly as possible of the same weight. The animals were individually caged and placed on the experimental diets shortly after weaning. Up to the time of being placed on the experiment, the rats had received the stock ration of the breeding colony.

TABLE 1  
*Feed formulas used*

Ingredient	Group I Per cent	Group II Per cent	Group III Per cent
When feeding tannic acid and theobromine			
Whole milk powder	63.00	61.74	62.83
Cane sugar	37.00	36.26	36.90
Tannic acid	0.00	2.00	0.00
Theobromine	0.00	0.00	0.27
When feeding cocoa powders			
Whole milk powder	63.00	52.90	52.90
Cane sugar	37.00	31.10	31.10
Cocoa (12.15% tannic substances)	0.00	16.00	0.00
Cocoa (2.67% tannic substances)	0.00	0.00	16.00
When feeding concentrated extract of cocoa			
Fluid whole milk	87.00	87.00	
Cane sugar	5.00	5.00	
Water	8.00	0.00	
Extract of cocoa (7.54% T. S.)	0.00	8.00	

NOTE.—Fe, Cu, and Mn were fed to all groups as explained in the text.

Three diets were compounded as shown in table 1, and fed one to each rat in the groups of three individuals, there being eight individuals on each

of the three diets. The rats were fed in accord with the principle of paired feeding, however, in this instance, triplets instead of pairs. Group I was the control group, which was fed the basal diet consisting of whole milk powder and cane sugar. Group II was fed the basal diet with the addition of 2 per cent crystalline tannic acid (C. P. Baker's Analyzed); and group III, the basal diet with the addition of 0.27 per cent theobromine (Eastman Kodak Co.). The amount of tannic acid and theobromine in the diets for groups II and III was equal to that in a ration containing 16 per cent cocoa powder which contains 12.15 per cent tannic substances and 1.7 per cent theobromine. A ration of approximately such a composition was fed in the next experiment.

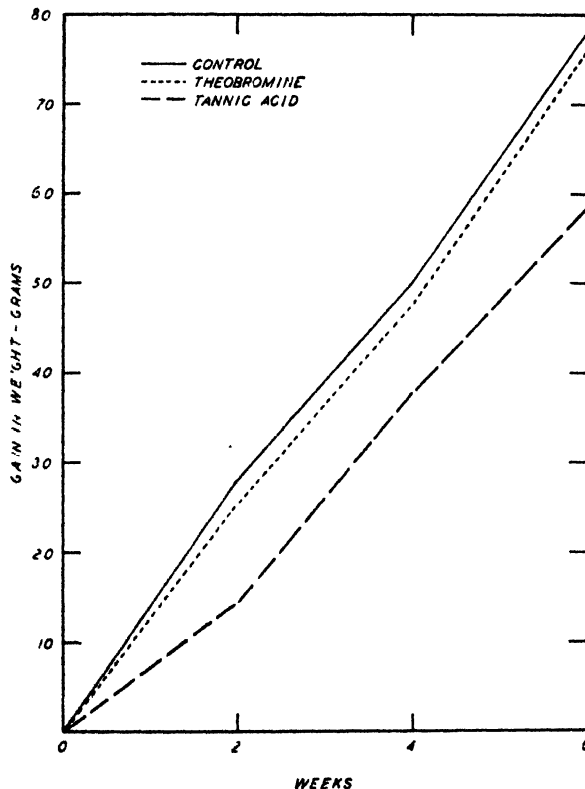


FIG. 1. Comparative growth of rats when feeding tannic acid and theobromine in a basal diet.

Iron, copper, and manganese were fed to all animals in amounts recommended by Elvehjem *et al.* (1). The mineral solutions were added to a 10 per cent sugar solution immediately before feeding, and the total volume of solution fed to each rat daily was 2 cc. The rats had water before them all the time.

Each rat in a group of three was fed the same amount of milk powder

and sugar so that the only variable in the ration was the tannic acid and theobromine. The animals were fed every other day and the amount of feed consumed determined. The quantity of food given to each group of three rats was determined by the quantity consumed by the individual eating the least within the group. Usually the animals receiving the tannic acid diet determined the food intake in all groups. The food was weighed into a

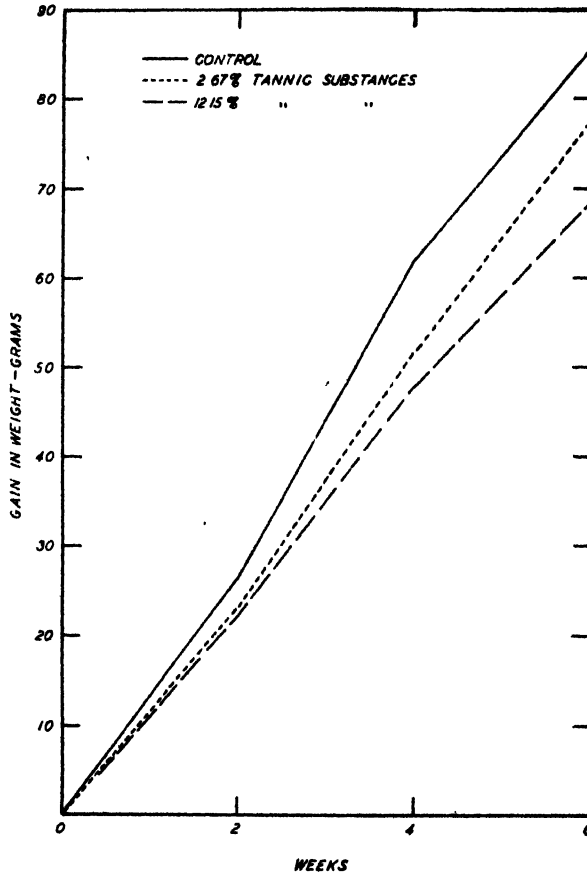


FIG. 2. Comparative growth of rats when feeding cocoa powders of varying tannic substances content in a basal diet.

porcelain feed cup of approximately 75 cc. capacity, which was set in a metal cup and held in place by a metal cover. This arrangement reduced the spillage to a minimum.

The rats remained on the experimental diets for six weeks and were weighed weekly during this time. Blood hemoglobin determinations were made after two and six weeks. The Newcomer method with a Klett colorimeter was used and the solutions were read against a standard Newcomer glass disk.

TABLE 2

*Average daily gain in weight and feed consumption during the six week period when feeding tannic acid and theobromine in a basal diet*

Ration	Gain in weight <i>gms.</i>	Decrease in weight over control	<i>Per cent</i>	Basic ration intake	Tannic acid intake	Theobromine intake
Group I*—Control	1.85			<i>gms.</i>	<i>gms.</i>	<i>gms.</i>
Group II*—Tannic acid	1.38	0.47 ( $\pm 0.09$ ) †	25.4	6.07	0.0	0.00
Group III*—Theobromine	1.81	0.04 ( $\pm 0.07$ )	2.1	6.07	124.0	0.00
				6.07	0.0	16.40

\* Eight rats in each group.

† Standard error of mean difference between control and tannic acid and theobromine.

TABLE 4

*Average daily gain in weight and feed consumption during the six week period when feeding cocoa powders, varying in tannic substances content, in a basal diet*

Ration	Gain in weight <i>gms.</i>	Decrease in weight gain over control	<i>per cent</i>	Basic ration intake	Cocoa intake	Tannic substances intake
Group I*—Control				<i>gms.</i>	<i>gms.</i>	<i>gms.</i>
Group II*—Cocoa high in tan- nic substances	2.04	0.42 ( $\pm 0.12$ ) †	20.6	6.26	0.00	0.00
Group III*—Cocoa low in tan- nic substances	1.62			6.26	1.19	144.58
	1.84	0.20 ( $\pm 0.10$ )	9.8	6.26	1.19	31.77

\* Seven rats in each group.

† Standard error of mean difference between control and cocoa powders of varying tannic substances content.

Since the sexes were equally distributed for all rations, the data are presented in figure 1 and table 2 as averages for male and female. The data in table 2 show that the eight rats receiving the control diet gained an average of 0.47 grams more per day than those receiving the tannic acid, and only 0.04 grams more per day than those receiving the theobromine. The standard error being 0.09 and 0.07 grams, respectively, the difference is statistically highly significant for the tannic acid and non-significant for the theobromine.

Frequently throughout the six week experimental period the rats fed tannic acid suffered a peculiar diarrhea. They were somewhat paler and not equal in appearance and well-being to the control rats and those fed theobromine. All three groups of rats had normal content of blood hemoglobin after being on the experimental diets for two and six weeks.

*Feeding cocoa powders with a high and a low content of tannic substances.* The purpose of this experiment was to determine whether or not the tannic substances in cocoa have the same toxicity as pure crystalline tannic acid. Studies on the toxicity of theobromine were not followed up because feeding the pure alkaloid in the first experiment failed to produce in the rat toxic symptoms which were sufficiently outstanding and definite to provide a base for a plan of experimentation.

The analysis of the two cocoa powders used for this study is given in table 3. The two cocoa powders were chosen after analysis of eighteen commercial powders because they differ greatly in tannic substances content, and yet are similar in many other respects.

TABLE 3  
*Analysis of cocoa powders*

Constituents	Cocoa high in tannic substances	Cocoa low in tannic substances
	<i>Per cent</i>	<i>Per cent</i>
Moisture	5.40	4.32
Protein*	22.62	20.31
Theobromine + caffeine	1.87	2.34
Fat	11.23	10.32
Fiber	5.25	5.33
Other N-free matter	35.50	43.11
Ash	5.98	11.60
Tannic substances†	12.15	2.67

\* Nitrogen (minus theobromine and caffeine nitrogen)  $\times 6.25$ .

† Ulrich's method as modified by Kuzmeski and Mueller (3).

Twenty-four young rats, twelve males and twelve females, were used in this experiment. The animals were divided and grouped as in the first experiment and unless otherwise specified the same experimental procedure was used. Three diets were prepared as shown in table 1. Group I was the control group which was fed the basal diet consisting of whole milk powder

and cane sugar. Group II was fed a diet containing the same constituents as the basal diet, but in addition 16 per cent of a cocoa powder that contained a large amount (12.15 per cent) of tannic substances. The diet for group III had the same constituents as for group II, except that the cocoa contained a small amount (2.67 per cent) of tannic substances. On a fluid milk basis the diets for groups II and III contained approximately 3.6 per cent by weight of cocoa powder, and 7 per cent of cane sugar. In most instances for this experiment, the rats that were fed cocoa high in tannic substances determined the food intake in all groups.

There were eight animals on each of the three diets at the beginning of the experiment. Since one male rat which received the cocoa high in tannic substances died after five weeks, from an unknown cause, the remaining two animals in that group are not included in the data.

The data are presented in table 4 and in figure 2, and show that the seven rats receiving the control diet gained an average of 0.42 grams more per day than those receiving the cocoa powder with a high content of tannic substances, and 0.20 grams more per day than those receiving the cocoa powder with a low content of tannic substances. The standard error being 0.12 and 0.10 grams, respectively, the difference is statistically significant for the cocoa high in tannic substances, and almost statistically significant for the cocoa low in tannic substances. Therefore, the toxicity of the latter to rats is questionable.

It should be noted that the diminution in growth of rats fed cocoa high in tannic substances is 20.6 per cent as compared to a 25.4 per cent decrease when crystalline tannic acid was fed in the first experiment. Animals fed cocoa high in tannic substance content were not equal in appearance and well-being to the animals which were fed cocoa low in tannic substances. However, these differences were not as great as one would expect when contrasting rate of growth. No significant differences in hemoglobin content of blood of the rats receiving the three diets were noted after two, four, and six weeks.

*Feeding a concentrated extract of cocoa powder which is free from tannic substances.* It was desired to test the toxicity of a commercial cocoa powder entirely free of tannic substances. Since such a cocoa powder could not be found, attention was given to extracts of cocoa. Some commercial chocolate-flavored syrups are flavored with an extract of cocoa in place of cocoa or chocolate. Such an extract was obtained from a company manufacturing a commercial chocolate syrup, and upon analysis the tannic substances and theobromine content were found to be practically nil.

Two rations were compounded as given in table 1, and were fed one each to a group of eight rats. The paired feeding method was used and other experimental procedures were similar to those used in the two previous experiments. Group I was the control, which was fed pasteurized whole



milk (approximately 4 per cent butter fat) and cane sugar. Water was added to offset the amount of cocoa extract added to the diet for group II. The diet for group II contained the same constituents as the basal diet, except that 8 per cent by weight of cocoa extract, containing 7.54 per cent solids, was substituted for an equal weight of water. One gram of the concentrated extract contained the extractable material from 0.48 grams of cocoa powder. Thus, the amount of the extract added to the milk was equal to the extractable material from cocoa powder when the latter makes up 3.8 per cent of the whole milk plus sugar ration. The animals were fed daily and the Fe, Cu, and Mn solutions were added to the milk immediately before feeding.

Data in table 5 show that the extract of cocoa was not toxic to rats. Although the percentage gains were slightly greater for the extract than for the control, the difference is not great enough to be statistically significant. Both groups of rats were equal in appearance and general well-being.

TABLE 5

*Average daily gain in weight and feed consumption during the ten week period when feeding concentrated extract of cocoa in a basal diet*

Ration	Gain in weight	Increase in weight gain over control		Basic ration intake	Cocoa extract intake
	<i>gms.</i>	<i>gms.</i>	%	<i>gms.</i>	<i>gms.</i>
Group I*—Control	1.55			34.78	0.00
Group II*—Conc. cocoa extract	1.62	0.07 ( $\pm 0.08$ )†	4.5	34.78	3.28

\* Eight rats in each group.

† Standard error of mean difference between control and concentrated extract of cocoa.

#### DISCUSSION

Investigations of tannins and their significance in food materials other than cocoa may be relevant to results obtained from this study. Lease and Mitchell (4) found the average tannic acid content of four Lespedeza hays to be 7.42 per cent, and they associate this substance with the toxic effect which this hay sometimes has on animals consuming it. These investigators fed various levels of tannic acid, gallic acid, and Lespedezas to white rats. They found that albino rats were able to tolerate 5 per cent of tannic acid mixed in a good ration, but higher levels resulted in decreased growth and the rats were pale and free from the characteristic pink of the normal animals. They also found that the hemoglobin levels of the blood of rats fed crystalline tannic or gallic acid were often 50 to 60 per cent lower than normal. Also gallic acid was found to be more toxic than tannic acid and the tannic acid of the Lespedeza less toxic than pure crystalline tannic acid.

The 25 per cent decrease in growth rate when 2 per cent crystalline tannic

acid was added to the ration in this study is not in accord with the findings of Lease and Mitchell, namely, that rats can tolerate 5 per cent crystalline tannic acid in the diet. The preliminary report by these investigators does not include the feeding procedure used nor the composition of the ration to which the tannic acid was added. Therefore, it is difficult to account for the apparent discrepancy. In this study the tannic substances in cocoa were found to be slightly less toxic than pure crystalline tannic acid. Likewise, Lease and Mitchell found that the tannic acid of the *Lespedeza* was less toxic than pure tannic acid. These results indicate that the tannic substances in cocoa are partly detoxified by chemical combination with other constituents. The results obtained when two cocoa powders were compared, one low and the other high in tannic substances, show that the toxicity is not directly proportional to the amount of tannic substances present, as determined by the modified Ulrich method. This may be due to the probability that the various tannic substances in cocoa are not equal in toxicity, while the analysis includes both the toxic as well as the non-toxic substances. Speculation on this point seems futile until more is known about the various tannic substances in cocoa.

Although the animals which were fed crystalline tannic acid and cocoa high in tannic substances appeared to have a somewhat paler skin, yet no differences of any significance were noted in the hemoglobin content of the blood. The lack of any reduction in hemoglobin may be due to adding iron to all of the rations. No doubt sufficient excess of iron was present to prevent any marked reduction in hemoglobin by the tannic acid or tannic substances in the diet. Lease and Mitchell also report that both iron and protein to some extent counteracted the toxicity of crystalline tannic acid fed to rats.

Ringrose and Morgan (6) fed day-old chicks a ration containing 2 per cent tannic acid, which resulted in reduced growth but did not cause any mortality. However, the reduced growth was the result of a reduced feed consumption.

#### SUMMARY AND CONCLUSIONS

1. Pure theobromine was non-toxic to albino rats when the ration contained 0.27 per cent of this alkaloid.
2. Pure crystalline tannic acid was toxic to rats when the ration contained 2 per cent of this substance.
3. A cocoa powder containing 12.15 per cent tannic substances was more toxic to rats than a cocoa powder containing only 2.67 per cent tannic substances when the two cocoa powders had approximately the same theobromine plus caffeine content. The tannic substances from cocoa were less toxic than pure crystalline tannic acid.

4. A concentrated extract of cocoa was non-toxic to rats when the ration contained 8 per cent.

5. The hemoglobin levels of the blood of rats fed theobromine, crystalline tannic acid, and cocoa powder containing varying amounts of tannic substances, did not vary from the normal enough to be of any significance.

6. It is concluded that the toxicity from cocoa can be greatly reduced by selecting a cocoa or chocolate which is low in tannic substance, or preferably using an extract of cocoa as the flavoring material when feasible.

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## STUDIES OF LIPASE ACTION

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### IV. THE INACTIVATION OF MILK LIPASE BY HEAT

In a previous paper (6), a method was described for following the lipolysis of milk fat by direct titration of the fatty acids in the fat itself. This method seemed particularly suitable for a study of lipolysis in butter as related to time and temperature of pasteurization, since it yielded definite numerical data, and the experiment could be continued for a long period of time, thus revealing the presence of very small amounts of lipase.

Several investigators have studied the thermal destruction of lipase (12, 10, 13, 3, 11, 5, 9) but, in many cases, their methods have been indirect and less sensitive than the method now available.

#### EXPERIMENTAL

Six series of samples were prepared on six successive days. In each case, the milk was obtained in the morning from the same group of eleven cows which were known to produce milk high in lipase activity. The mixed milk was separated, without cooling, at 91° F. (33° C.) to yield a cream containing 20 per cent of fat. This cream was heated in a water bath to a predetermined temperature (different on each day) and portions were removed after various periods of holding. These portions were stored in ice water for twenty-four hours, and then churned at 58° F. (14° C.) in a battery of motor driven churns immersed in a constant temperature bath.

The butter samples were washed and stored unsalted, in sterile jars. Samples were removed periodically for the determination of free fatty acids. At the end of 58 weeks, the experiment was concluded and Professor E. S. Guthrie was asked to score the butter samples.

The storage temperature was not uniform during the entire period. During the first six weeks and the last eighteen weeks, the butters were held at 0° to 8° F. During the remainder of the period they were held at 28° to 32° F.

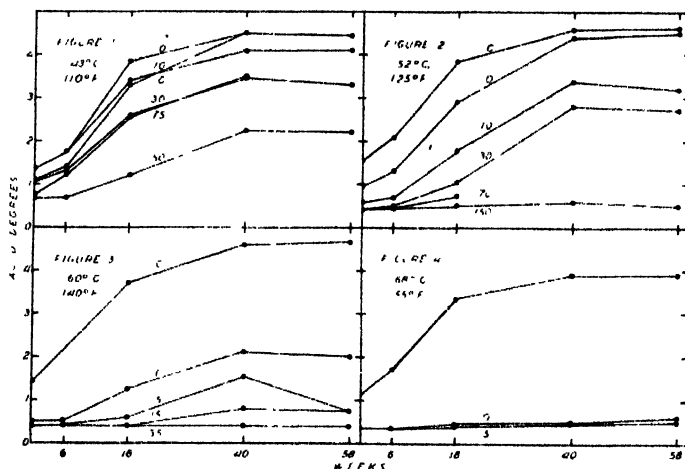
#### DISCUSSION

Figures 1 to 4 show the progress of lipolysis in these samples. The data for the higher temperatures, 170° F. (77° C.) and 180° F. (82° C.), are omitted because no appreciable lipolysis occurred in any of the samples heated to these temperatures. The slight increase (about 0.2 acid degrees) observed in all of the highly heated samples is possibly due to the normal hydrolysis of fat in contact with water, even in the absence of an enzyme.

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These data indicate that the destruction of lipase is quite rapid at 155° F. (68° C.). At 140° F. (60° C.), 125° F. (52° C.), and at 110° F. (43° C.), the destruction is slower, and clearly related to the time of exposure to heat. These results are in substantial agreement with those of other investigators.

The curves of figure 1 show an acceleration in lipolysis after six weeks of storage and a cessation after forty weeks. This is apparently due to the change in storage temperature at these times. The data indicate that lipase in butter is inactive at very low temperatures (0° to 8° F.) but active at 28° to 32° F.



FIGS. 1-4 The relation between the temperatures of preheating (°F.) and the length of time (0, 5, 10 . . . 150 min.) the cream was held at various temperatures and the subsequent lipolysis of butter during storage. Storage temperature from the 6th to 40th week was 28° to 32° F.; at other times 0° to 8° F. (Symbol "C" on the graphs indicates unheated samples.)

The initial acid degrees of the unheated samples are above the normal values for fresh milk (6). These high values are due to the agitation during churning, since agitation is known to accelerate lipase action. The high initial values of many of the samples are due to the stimulating effect of agitation upon lipolysis (8). The liberation of free acid during churning had a marked effect upon the churning time (7). For example, in the case of the samples held at 125° F., the churning times were 80, 65, 50, 43, 45, and 43 minutes, while the initial acid degrees of the butters were 1.56, 0.94, 0.58, 0.42, 0.40 and 0.41, respectively.

Table 1 shows the relation between the time and temperature of holding and the acid degree and score of the butter after 58 weeks' storage. The rancid samples are indicated by italics. Some of the other samples were scored down because of a "musty" flavor. These data indicate that a rancid flavor appears in butter between acid degrees of 0.75 and 2.0. The lower

TABLE 1  
*The acid degrees and judges' scores after 58 weeks in storage*  
 Rancid samples are indicated by italicized scores

Holding temperature	Not heated	Heated, not held	Held 10 minutes	Held 30 minutes	Held 150 minutes
	<i>degree score</i>	<i>degree score</i>	<i>degree score</i>	<i>degree score</i>	<i>degree score</i>
110° F.	4.45 — 86	4.75 — 85	4.75 — 84	3.57 — 84	2.50 — 87
125° F.	4.62 — 84	4.50 — 84	3.20 — 84	2.70 — 84	0.50 — 91
			Held 5 minutes	Held 15 minutes	Held 35 minutes
140° F.	4.65 — 84	2.00 — 84	.75 — 88	.75 — 91.5	.40 — 92
155° F.	3.91 — 84	.59 — 90	.59 — 92	.50 — 92	.45 — 93
170° F.	4.70 — 85	.60 — 88	—	.62 — 88	.62 — 91
180° F.	3.89 — 87	.40 — 93	.45 — 92	.47 — 92	.47 — 92

value is believed to be nearer the threshold of rancidity as determined by flavor.

Data have been published recently which show no relation between fat acidity and organoleptic rancidity (4). However, in that study a variety of agents may have been responsible for the rancidity of different samples. In these experiments, involving a uniform substrate and only the enzymes of the milk itself, the two measures of lipolysis seem closely related.

#### SUMMARY

Butter samples were churned from fresh cream held at 110°, 125°, 140°, 155°, 170°, and 180° F., for periods of time ranging from 0 to 150 minutes. The rate of lipolysis during storage was measured by titration of free acids in the fat.

At 110° F., lipolysis was first activated and then reduced as the holding time was increased. The rate was reduced about  $\frac{2}{3}$  by holding 150 minutes.

At 125° F., the rate of lipolysis was reduced about  $\frac{1}{2}$  after 20 minutes (estimated) but it was still measurable after 150 minutes.

At 140° F., the rate of lipolysis was reduced more than half at zero holding time. The rate was measurable with a holding period of 15 minutes but not after 35 minutes.

At 155° F., the rate of lipolysis was scarcely measurable after zero minutes of holding.

#### V. THE EFFECT OF STORAGE TEMPERATURE UPON LIPOLYSIS IN BUTTER

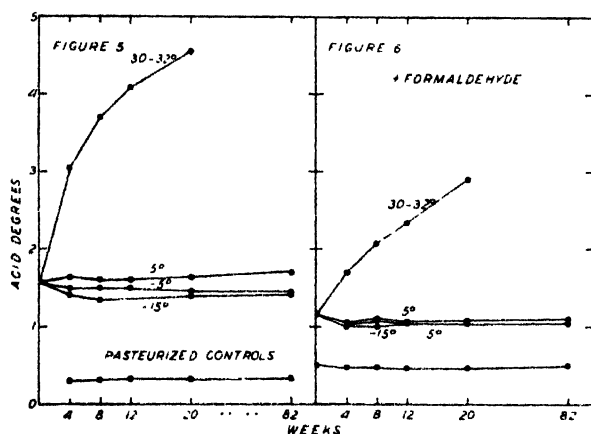
It has been reported that lipases may be active at temperatures as low as -30° C. (1). The rate of lipolysis is dependent upon the nature of the fat (1, 8), and possibly upon the nature of the enzyme. For that reason, it seemed worth while to test the action of the natural lipases of milk upon milk fat at such low temperatures as might be used for the storage of butter, to learn if their action continued at temperatures below 0° C.

#### EXPERIMENTAL

It was desired to study the formaldehyde sensitive (6) and the formaldehyde tolerant enzymes separately. Consequently, two groups of cows were selected from the University herd. The first produced milk relatively rich in the formaldehyde sensitive enzyme. (The extent of lipolysis during 24 hours was reduced 84 per cent by 0.1 per cent by volume of formalin.) The second group produced milk relatively rich in the formaldehyde tolerant enzyme. (Lipolysis was reduced only 44 per cent by 0.1 per cent by volume of formalin.) When formaldehyde was added to the milk of this second group of cows, subsequent lipolysis could be attributed entirely to the formaldehyde tolerant enzyme. Lipolysis in milk of the other group could be

attributed chiefly, though not entirely, to the sensitive enzyme. Unsalted butters were prepared from these two types of milk, with care to avoid bacterial contamination. Each lot of butter was divided and packed into sterile glass tubes. A number of samples of each of these butters was then stored in a commercial cold storage warehouse, at a series of temperatures, approximating  $-15^{\circ}\text{F.}$ ,  $-5^{\circ}\text{F.}$ ,  $5^{\circ}\text{F.}$ , and  $30-32^{\circ}\text{F.}$  At the same time, portions of the two milks were pasteurized at  $155^{\circ}\text{F.}$  for 30 min., and control samples of pasteurized butter were prepared and stored in the same way.

Samples of butter were removed from storage at intervals and the acid degree was determined. All samples showing any evidence of mold were discarded. The data are presented in figures 5 and 6. Due to an oversight,



FIGS. 5 and 6. The rate of lipolysis in unpasteurized butters stored at  $-15^{\circ}\text{F.}$ ,  $-5^{\circ}\text{F.}$ ,  $5^{\circ}\text{F.}$  and  $30-32^{\circ}\text{F.}$  FIG. 5. Butters prepared from milk in which the sensitive enzyme was predominant. FIG. 6. Butters prepared from milk in which the formaldehyde sensitive enzyme had been destroyed.

the control samples of raw butter were not pasteurized promptly. This accounts for the fact that the initial values recorded are higher than the values for the stored samples. Although this was unfortunate, it does not invalidate the remaining data.

The data for the pasteurized samples are not shown in detail, because all samples of each pasteurized butter yielded the same values (within experimental error) regardless of the time, or temperature of storage. It should be noted that the  $30-32^{\circ}\text{C.}$  series of pasteurized samples was discarded after 20 weeks because all of the remaining samples showed evidence of mold growth.

These data indicate that, in sweet cream butter, the natural lipase of the milk are not appreciably active at temperatures of  $5^{\circ}\text{F.}$  or lower. It seems probable that this sharp reduction in rate is due to the freezing of the water present in the sample. If that is the case, then the slight increase in acidity



which appeared during the first part of the storage period may be explained by assuming that the water dispersed through the fat does not freeze promptly, but remains in a super-cooled state for varying periods of time. Despretz and also Dufour have demonstrated that water may be cooled to  $-20^{\circ}\text{C}$ . ( $-4^{\circ}\text{F}$ .) without freezing (2). It seems quite likely that much of the water in the samples stored at  $30-32^{\circ}\text{F}$ . never did freeze during the storage period.

#### SUMMARY

Samples of unsalted sweet cream butter were stored at a series of temperatures ranging from  $32^{\circ}\text{F}$ . to  $-15^{\circ}\text{F}$ . for more than one year. The extent of lipolysis was measured at intervals by titration of the free acids in the fat. The data indicate that, in butter, lipolysis by the natural lipases of milk is inhibited at  $5^{\circ}\text{F}$ . or lower, though they are active at  $30-32^{\circ}\text{F}$ .

The data do not reveal any difference in the abilities of the formaldehyde tolerant and the formaldehyde sensitive enzymes to act at low temperatures.

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## STUDIES OF LIPASE ACTION. VI.

### THE EFFECT OF LIPOLYSIS UPON THE FLAVOR SCORE OF MILK

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In a previous paper (3) it was shown that the very early stages of lipolysis in milk could be followed by titrating the free acids in the fat. Since that time, a great deal of data has been accumulated in terms of fat acidity, and it seemed desirable to interpret these data in terms of flavor, if possible. In particular, we wished to know the "acid degree" at which rancidity could first be recognized as such and, second, we wished to learn the rate at which a judge would reduce the score of the milk as the acidity of the fat increased.

It should be noted that these experiments deal only with lipolysis caused by the natural enzymes of milk. They are not comparable with the experiments of others involving the lipases of bacteria, or molds, or the possible destruction of free fatty acids by microorganisms (1, 2, 5). On the other hand, it is believed that the changes reported here are comparable with those occurring in fluid milk on its way to market.

In this investigation it was desired to obtain judges' scores on a large number of samples of milk of different degrees of rancidity. In order to obtain these samples, the following plan was adopted. Three lots of milk were obtained at milking time from selected cows. The first lot was known to be very low in lipolytic activity, the second was moderately active, while the third was very active. Each of these lots of milk was sub-divided into three portions. One was pasteurized, the second was left in its natural state, while the third was activated by temperature treatment (4) to increase the rate of lipolysis. It was believed that this procedure would yield samples undergoing lipolysis at six different rates.

Eight one-pint samples from each lot of milk were stored at 0-5° C., and one bottle from each set was scored each hour. Immediately after the samples were scored, the remainder of each sample was pasteurized to prevent further lipolysis, and saved for analysis.

The scoring was done by Professor E. S. Guthrie and Mr. S. N. Friedman. In most cases, the samples were examined by both judges but at a few times only one judge could be present. The numbers on the samples gave the judges no indication of the relation between the samples of one set and the samples examined an hour earlier.

Before examining the data, it should be noted that all samples were less

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than nine hours old when tasted by the judges, and that the milk samples had been held at less than 5° C. during almost the whole of that time.

As the judges recorded their scores, they often added comments such as feedy, old, bitter, etc. Of the terms used, "slightly rancid," "bitter," "rancid," and "bitter-rancid" seemed to refer to some degree of lipolysis. A tabulation of the acidities of the samples described by these terms is shown in table 1. This tabulation shows that the threshold at which rancidity can be recognized is near an acid degree of 0.8. Of twenty-eight samples exceeding this value, only six escaped recognition as rancid; of twenty-seven samples having acid degrees between 0.4 and 0.79, only eight were recognized as rancid. The exact threshold value will, of course, depend upon the presence or absence of other flavors, and upon the individual judge. It may also depend upon the fat content and its composition.

TABLE 1

*The frequency with which certain terms were applied to milk containing fats of different rancidities*

Acid degrees	Term used			
	"Bitter-Rancid"	"Rancid"	"Slightly Rancid"	Other terms
0.0-0.39			1	54
0.4-0.79		1	7	19
0.8-1.19		3	2	6
1.2-1.59	4		1	
1.6-1.99	4	1		
2.0-2.39	2			
2.4-2.79	1			
2.8-3.19	3			
3.2-3.59	1			

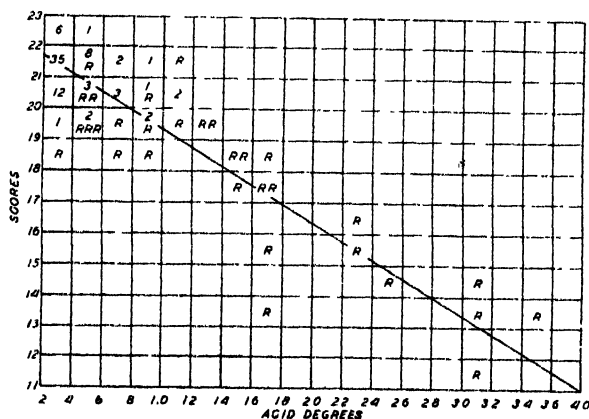


FIG. 1. The frequency with which different scores were assigned to milks containing fats of different acidities. (Samples falling on dividing lines were considered in lower group.) Symbols 1, 6 . . . 35 indicate number of samples escaped recognition as rancid; R indicates one rancid sample.

The relation between the scores reported by the judges and the acid-degree of milk-fat is shown in figure 1. The relationship between lipolysis and the score is quite apparent; in fact, for this particular pair of judges, the relationship appears to be linear. The method of least squares yielded the relationship: flavor score =  $22.32 - 3.00 \times$  acid degrees.

These data seem to indicate that judges may lower the score of milk, as a result of lipase action, even before the nature of the defect can be recognized. This seems of practical importance because the degree of lipolysis in some commercial milks (3) is such as to place them in this region where judges may cut their scores without being aware of the real reason for so doing.

It seems of interest to note that no sample having an acid degree above 1.4 received a flavor score above 19.

#### SUMMARY

A study was made of the relationship between the judges' scores and the "acid degrees" of raw milk within a few hours after milking.

The data indicate a threshold value for the recognition of rancidity at acid-degrees near 0.8. They also indicate that very slight degrees of lipolysis, such as is common in commercial market milk, may influence a judge's score without his being aware of the reason.

In the case of this particular pair of judges, and milk supply, statistical analysis shows that an increase of 0.33 acid degrees corresponded to a decrease of one point in the flavor score of the milk.

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# STUDIES OF LIPASE ACTION. VII. THE INFLUENCE OF THE RATE OF COOLING UPON THE SUBSEQUENT RATE OF LIPOLYSIS IN MILK STORED AT LOW TEMPERATURES

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In a previous paper (1), we reported that the rate of lipolysis in milk during storage at low temperatures was greatly influenced by the speed with which the milk had been cooled. By very rapid cooling, the rate of lipolysis during storage was reduced to a third of the value observed when the milk had been cooled more slowly.

Because of the possible value of this phenomenon in controlling rancid and bitter flavors in milk, it was subjected to further investigation. Our observations are reported here.

## METHODS

The extent of lipolysis in the different samples was measured by titrating the free acids present in the fat, using the same procedure as before (1). Our results are reported in acid degrees, that is the number of ml. of normal alkali required to neutralize the acid in 100 grams of fat. All samples were pasteurized before churning.

The tubular cooler, used for rapid cooling, consisted of a spiral tube immersed in 25 liters of crushed ice and water. The spiral was made from a 12 foot (3.66 meter) length of  $\frac{3}{8}$  inch (.95 cm.) outside diameter aluminum tubing. The portion of the tube immersed in the bath had a capacity of 120 ml. Unless stated otherwise, samples cooled in air, or in ice water, were cooled in  $\frac{1}{2}$  pint bottles without agitation.

## EXPERIMENT I

As a check on our previous work, a sample of mixed milk, representing a herd of about 80 cows, was taken at milking time and divided into four parts. The first, a control sample, was pasteurized at once, the second was cooled in air at 0° C., the third was cooled in crushed ice and water, and the fourth was cooled to 5° C. with the tubular cooler. When the temperature of each sample reached 5° C., it was transferred to a constant temperature cabinet and held at 5° C. for 48 hours.

The relative speeds of lipolysis are shown by the *increase* in acid degrees of the three samples: air cooled, 0.41; water cooled, 0.37; tube cooled, 0.06. These results are in agreement with our previous observations, though the milk shows less lipase activity.

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In other experiments we have observed that either the water-cooled or the air-cooled milk may show the greater degree of lipolysis. We suspect that there may be a critical rate of cooling which results in a maximum degree of lipolysis. This rate may depend upon the nature of the milk. This matter has not yet been studied.

#### EXPERIMENT II

Since rapid cooling seemed to retard lipolysis in raw milk, it was thought worth while to study several rates of cooling in the high speed range.

The experiment presented some difficulties. At first, we proposed to raise the coil part way out of the cooling bath, and then reduce the rate of flow of the milk until we obtained the same final temperature as before. However, this procedure had to be rejected because it would not reduce the rate of cooling of individual particles of the milk. The reduction in speed of flow would almost exactly compensate for the reduction in length of the refrigerated portion of the coil, and the time required for an individual particle of milk to pass over the cold surface would not be changed.

In order to reduce the rate of cooling, we finally coated the tube with a film of paraffin. We were able to secure several rates of cooling by applying coatings of different thicknesses. In each case, the rate of flow was so adjusted that the milk leaving the coil had a temperature of  $2^{\circ}\text{C.} \pm \frac{1}{2}^{\circ}$ . These samples were stored at  $5^{\circ}\text{C.}$  for 48 hours. Samples of the same milk were cooled in air and in water for comparison with those cooled more quickly. The data are shown in table 1.

TABLE 1

*The relation between the speed of cooling and the extent of lipolysis during 48 hours' storage at  $5^{\circ}\text{C.}$*

Method of cooling	Time from $33^{\circ}$ to $2^{\circ}\text{C.}$	Increase in acid degree
Tube	26'''	0.15
Tube	35'''	0.15
Tube	1' 42'''	0.16
Tube	2' 35'''	0.21
Tube	2' 45'''	0.21
Bottle in water	25'	0.73
Bottle in air	140'	0.55

\* Actually the time required to discharge a volume equal to the capacity of the coil.

These data are in agreement with all previous observations that the rate of lipolysis is reduced when the milk is cooled very rapidly, and they suggest that a greater reduction might be obtained by a further increase in the rate of cooling.

In order to test this, a new coil was constructed from smaller tubing. The two coils were mounted side by side in the same tank of crushed ice and water. The rates of flow were so adjusted that the milk leaving each coil had

a temperature of  $2^{\circ}\text{C.} \pm \frac{1}{2}^{\circ}$ . The average cooling time was calculated from the known capacity of the coil and the time required to collect a  $\frac{1}{2}$  pint sample.

Three separate lots of milk were obtained at milking time and parts of each were cooled in each coil. At the same time,  $\frac{1}{2}$  pint samples were cooled in air and in water for purposes of comparison. The data are shown in table 2.

TABLE 2

*The relation between the speed of cooling and the extent of lipolysis during 48 hours' storage at  $5^{\circ}\text{C.}$*

	Milk No. 1	Milk No. 2	Milk No. 3
Cooled in water			
Minutes from $32^{\circ}\text{--}2^{\circ}\text{C.}$	25	25	25
Increase in acid degree	2.11	1.30	0.77
Cooled in air			
Hours from $32^{\circ}\text{--}2^{\circ}\text{C.}$	2	2	2
Increase in acid degree	1.34	0.33	1.39
Cooled in large tube			
Seconds from $32^{\circ}\text{--}2^{\circ}\text{C.}$	35	32	30
Increase in acid degree	0.99	0.19	0.23
Cooled in small tube			
Seconds from $32^{\circ}\text{--}2^{\circ}\text{C.}$	10	8.5	8.5
Increase in acid degree	0.84	0.14	0.12

## EXPERIMENT III

In the experiments reported hitherto, we used natural milk, that is, milk which had not been activated by temperature changes (2). It seemed worth while to determine whether the speed of cooling was a factor in the activation process.

Since "activation" consists of 1. cooling the milk, 2. warming the milk, and 3. recooling the milk, there are two steps in the process where the rate of cooling might be important. In order to study both of these, the following procedure was used.

Approximately thirty liters of warm mixed milk was collected at milking time and divided into three equal parts. One was cooled at  $5^{\circ}\text{C.}$  by passing it through the larger coil, the second was cooled in an aluminum can immersed in ice water, the third was cooled in an aluminum can in air at  $0^{\circ}\text{C.}$  After cooling, each lot was warmed to  $30^{\circ}\text{C.}$  and samples from each lot were cooled in air, and in water. These samples were stored at  $5^{\circ}\text{C.}$  for 48 hours and then examined. The increase in free fatty acid during storage is shown in figure 1. Part A shows that, regardless of the method used for the first cooling, the rate of lipolysis was practically the same for all samples cooled in air after warming to  $30^{\circ}\text{C.}$  Part B shows that, regardless of the method used for the first cooling, the rate of lipolysis was approximately the same for all samples cooled in water after heating to  $30^{\circ}\text{C.}$  A comparison of parts



A and B shows a small difference in favor of the air cooling. This small difference has been observed in practically all of our experiments.

In order to show more clearly the effect of rate of cooling during the final stage of the activation process, one lot of milk was pre-cooled to 5° C. in the tube, warmed to 30° C. and then parts of it were cooled by all three methods. The samples were examined after 48 hours' storage at 5° C. The increases in

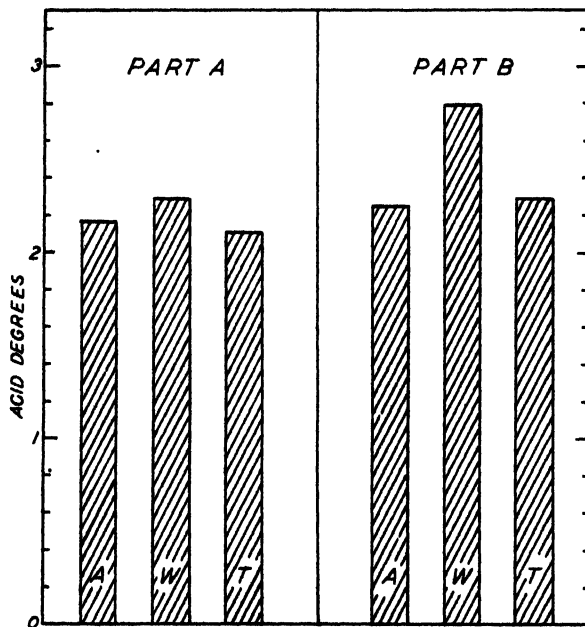


FIG. 1. The effect of the method of precooling upon the rate of lipolysis after temperature activation. Part A, final cooling in air; part B, final cooling in water. (A, pre-cooled in air; W, pre-cooled in water; T, pre-cooled in tubular cooler.) The chart shows increases in acid degrees during 48 hours at 0° C.; the initial values have been subtracted.

fat acidity were: Cooled in air, 2.11°; cooled in water, 2.29°; cooled in tube 0.23°. These data are similar to those obtained with unactivated milk but they are much greater in magnitude. They suggest that rapid cooling is even more effective in retarding lipolysis in activated milk than in natural milk.

#### EXPERIMENT IV

Our experiments have shown that lipolysis at low temperatures can be retarded by cooling rapidly from about 33° C. (as the milk reached the laboratory) to about 5° C. We wished to learn whether it is necessary to cool rapidly throughout this entire range, or whether there is only a narrow range of temperatures in which rapid cooling is essential.

This problem was resolved into two parts: (1) is there an upper range of temperatures where the rate of cooling has little effect, and (2) is there a

lower range of temperatures where rate of cooling has little effect. The second question was studied first.

A supply of fresh warm milk was cooled rapidly from 33° C. to various lower temperatures by passage through the tubular cooler. Each of these samples was subdivided and the cooling to 5° C. was finished more slowly. One part was cooled in water, the other in air. The samples were examined after 48 hours, and the data are shown in figures 2-A and 2-B respectively.

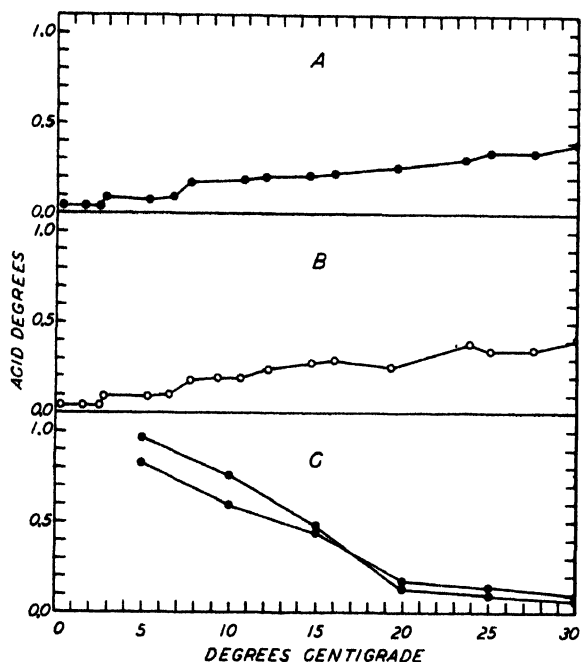


FIG. 2. Parts A and B, lipolysis during 48 hours at 5° C. in normal milk samples cooled quickly to the indicated temperatures and then slowly (in water -A, or in air -B) to the storage temperature; part C, lipolysis during 48 hours' storage at 5° C. in normal milk samples cooled slowly to the indicated temperature and then quickly (tubular cooler) to the storage temperature. The chart shows *increases* in acidity; the initial values have been subtracted.

Within experimental error, the data yield a straight line and cannot be regarded as evidence for a low temperature region where the rate of cooling is unimportant.

In order to answer the other question (Is there an upper range of temperatures where slow cooling is not detrimental?) a supply of fresh warm milk was cooled slowly to various temperatures and then the cooling was completed rapidly with the tubular cooler. The slower cooling was performed by placing the aluminum container in ice water. As usual, the samples were examined after 48 hours at 5° C.

The data for two samples, shown in figure 2-C, reveal that slow cooling in the range from 30° C. to 20° C. is not detrimental. The critical range through which the milk must be cooled rapidly to retard lipolysis extends downward from 20° C.

#### EXPERIMENT V

The milk used in experiment IV was not activated. The results of experiment III had indicated that the rate of cooling was more important in the case of activated milk. For that reason, experiment IV was repeated with activated milk to determine the critical cooling range for milk of this type.

In order to locate the lower limit of the critical cooling range, about 10 liters of milk was cooled in ice water to 10° C., warmed to 30° C. and then parts of it were cooled rapidly in the coil to various temperatures. The cooling was finished more slowly by cooling part of each sample in ice water and part in air at 0° C. These samples were examined after 48 hours' storage at 5° C.

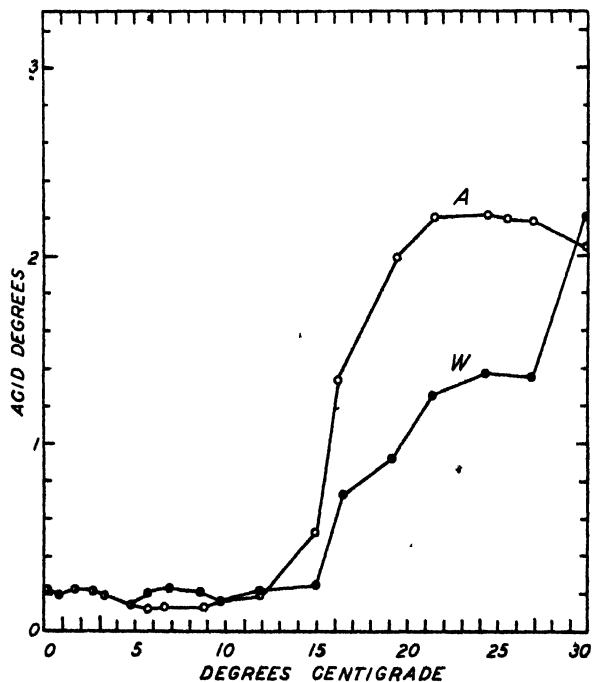


FIG. 3. Lipolysis during 48 hours at 5° C. in activated milk samples cooled quickly to the indicated temperature and then slowly (in air -A, or in water -W) to the storage temperature. The chart shows *increases* in acid degrees.

Figure 3 shows that, in activated milk, the rate of cooling in the range below 12° C. is of much less importance than the rate of cooling in the range

just above that temperature. The importance of cooling rate in this critical region is shown, also, by the spread between the values for the air-cooled and water-cooled samples.

In order to locate the upper limit of the sensitive range, a can of fresh warm milk was cooled slowly in ice water and, at various temperatures, samples were removed which were cooled quickly to  $2^{\circ} \pm \frac{1}{2}$  by means of the coil. The data obtained with two different lots of milk are shown in figure 4. They are quite similar to those obtained with unactivated milk

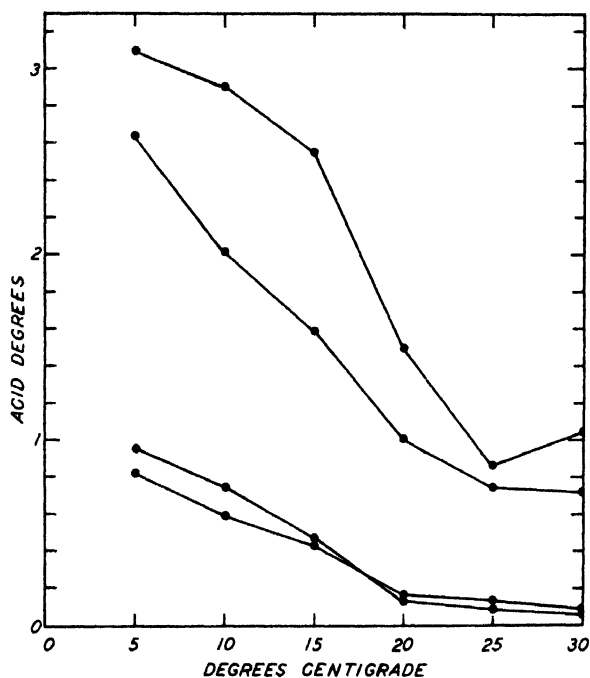


FIG. 4. Lipolysis during 48 hours' storage at  $5^{\circ}$  C. in activated milk (two upper lines) and normal milk (two lower lines) cooled slowly (in water) to the indicated temperature and then quickly (tubular cooler) to the storage temperature. Only the increase in acidity is shown; initial values have been subtracted.

though the upper limit of the critical cooling range appears to be  $25^{\circ}$  C. instead of  $20^{\circ}$  C.

#### SUMMARY

The rate of lipolysis in milk stored at low temperatures depends upon the rate at which the milk was cooled before the storage period.

To secure a minimum rate of lipolysis, the cooling time should be reduced to a few seconds.

There is a critical temperature range in which the rate of cooling is most

important. The upper limit of this range is approximately 20°–25° C. The lower limit is approximately zero in the case of natural milk, and approximately 10° C. in the case of temperature-activated milk.

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# THE RELATION OF THE USE OF CERTAIN ANTIOXIDANTS AND METHODS OF PROCESSING TO THE KEEPING QUALITY OF POWDERED WHOLE MILK\*

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The manufacture and utilization of dry milk has greatly increased in recent years as shown by the following production figures of the U. S. Department of Agriculture for the United States:

	1921	1938
Pounds of powdered whole milk	4,242,000	21,496,000
Pounds of powdered skim milk	38,546,000	4,942,000,000

Because milk in its natural form is such a perishable product, the commercial possibilities of dried milk products of good keeping quality have attracted the attention of dairy investigators for a number of years. The use of powdered whole milk and cream has been limited somewhat by certain problems connected with the satisfactory preservation of these products. For example, under certain conditions of storage powdered milk loses its original fresh flavor, decreases in solubility and frequently darkens in color. The most important off-flavor that may develop during storage of dried milk is one involving a chemical change in the butterfat and is termed tallowy or oxidized. The primary purpose of this study was to determine to what extent the antioxidants that have been found to retard or prevent the development of the oxidized flavor in fluid milk products would produce similar results in powdered whole milk. At the same time the relation of certain other factors such as type of container, per cent of moisture, temperature of preheating, and temperature of storage, to the keeping quality of the powdered milks was determined.

## REVIEW OF LITERATURE

A great many investigators have studied the problem of oxidized or tallowy flavor in dairy products and the factors pertaining to its development. The terms rancidity and tallowiness have been confused in the literature. Rancidity is a result of the hydrolysis of some of the volatile fatty acids, while tallowiness in dairy products is considered by most investigators (11) to be a result of an oxidation of the fatty substances present.

It has been shown by Holm, Greenbank and Deysher (9) that the clarification of milk before drying results in a definite improvement of the keeping quality of the powder. These same investigators explained the resis-

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tance of skim milk powder to oxidation as the result of the clarification the milk receives during separation. They further state that Supplee (16) reported an increase in resistance to oxidation with an increase in fat content and explain this effect on the centrifugal separation of the cream used in standardizing the milk.

Holm, *et al.*, also suggest that higher than ordinary pasteurizing temperatures (83–85° C. for 30 minutes) improves the keeping quality of whole milk powder. Their results also indicate that there is an improvement in the keeping quality of milk powder when the milk has been homogenized.

Because tallowy flavor is a defect resulting from oxidation of milk fat, it is natural to assume that powders made from whole milk are more susceptible to oxidation than those from skim milk. However, Supplee (16) shows that cream powders were much less susceptible to oxidation than powders with a lower fat content. These results do not agree with those obtained by Holm, Greenbank and Deysher (8).

Experiments by Supplee (16) have demonstrated that the addition of copper salts to milk before drying results in an early manifestation of tallowiness.

Factors such as air, light, temperature and moisture, known to be important in the development of tallowy flavor, have been given consideration in studies on the storage conditions and type of container used in relation to the development of this flavor defect. Holm and Greenbank (7) were able to show a decrease in the rate of oxidation of butter fat when the amount of oxygen in the storage container was reduced. They also point out that the storing of milk powder in inert gases will retard but not prevent the development of tallowiness.

According to the early research of Greenbank and Holm (5) the presence of moisture may retard the rate of oxidation of butterfat at ordinary storage temperatures. Chemical studies of milk fat by Supplee (16), Supplee and Bellis (17), and Holm and Greenbank (4, 10) indicate that oxidation may proceed at an equal rate in high and low moisture powders. From later work Holm, Wright and Greenbank (10) concluded that a high moisture content greatly increases the rate of oxidation, especially at high storage temperatures.

Dahle and Palmer (1) studied the keeping quality of milk powder at different temperatures and found that there was little difference in the powders stored at 4° C. and those stored at 20° C. but there was a marked difference at 37° C. Supplee (16) does not entirely agree with the results of Dahle and Palmer. He found that partially skim milk powder would not develop tallowiness for 18 months at 0° C. but at 20° C. the flavor was noticeable in 5 to 6 months. Holm, Wright and Greenbank (10) found there was no appreciable improvement in keeping quality unless temperatures of below 0° C. are used and that the rate of deterioration goes up rapidly at temperatures above 0° C.

Emery and Henely (2) reported that when lacquered metallic containers were used there was a marked improvement in resistance to oxidation as compared to the plain metal container. Dahle and Palmer (1) did not find any improvement in the keeping quality of milk powder when stored in lacquered tin. They did, however, find that "doubletite" containers prevented the discoloration of the powder at 37° C. and greatly improved the quality at all temperatures.

Supplee (16) and Supplee and Bellis (17) found that when the moisture content was maintained between 3 and 5 per cent there was little change in solubility during storage. Work by Dahle and Palmer (1) indicates that storage at high temperatures results in a marked decrease in solubility. Sharp, Doob and Willmann (14) confirm the results of Supplee, *et al.* and indicate that the browning and marked decrease in solubility of powders is related to a combined effect of moisture and storage temperature. They suggest that a reaction between the lactose and the casein results in the defect.

The effect of antioxidants as a preventive of oxidation in products containing fat have been studied by a number of investigators. Moureau and Dufraisse (12) found that traces of pyrogallol and hydroquinone were effective in preventing oxidation. Grettie (6) found that small amounts of gum guaiac retarded the oxidation of lard and that the antioxidant properties were carried over in bakery goods. He found that this gum produced no toxic effects. Greenbank and Holm (3) found that unsaturated polybasic aliphatic acids are effective as antioxidants and that maleic acid (0.01%) would retard the oxidation of butter oil stored at 42° C. Peters and Musher (13) observed that when 0.25–0.5 per cent of oat flour was added to milk before drying or mixed mechanically with its powder there was a substantial retention of fresh flavor and aroma and oxidation was retarded.

#### EXPERIMENTAL PROCEDURE

The investigation was divided into five sets of experiments as follows:

- a. Use of various antioxidants in whole milk powder.
- b. Variations in pasteurizing temperature.
- c. Variations in moisture content.
- d. Variations in type of container used.
- e. Variations in storage temperature.

All milk was received from the University Farm and processed in the stainless steel equipment of the University Creamery.

The milk used in preparation of the samples was processed as follows unless otherwise indicated. The raw milk was preheated to 90° F. and clarified at this temperature, then standardized to 4 per cent fat and pasteurized at 150° F. for 30 minutes. Following pasteurization the milk was



condensed, homogenized at 2,500 lbs. pressure, cooled over a surface cooler and tested for fat by the Mojonnier method.

The milk was dried on a double roll vacuum type drier, equipped with stainless steel pipes, fittings, knives, and rolls. The machine had a capacity of 2-3 pounds of powder per hour. The milk supply tank for the drier consisted of a large Pyrex glass funnel connected to the stainless intake pipe by means of rubber tubing.

In the pasteurizing temperature experiments, the milk was heated and homogenized in stainless steel equipment and then condensed in a laboratory condensing unit. The laboratory condensing equipment consisted of a 22 liter Pyrex glass flask immersed in a hot water bath and connected to a 2 liter suction flask by a 4-foot condenser. The suction flask was connected directly to a water pump and arranged so that vapors and condensate passed out the connecting line, through the water pump and into the drains.

In all experiments the percentage of antioxidants added is given on the basis of unconcentrated 4 per cent milk. The actual additions, however, were made to the concentrated product before drying.

At the time of drying, a solution of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  equivalent to 2 p.p.m. of copper, on the weight basis, was added to one-half of each lot of milk in experiments A and D. In experiments B, C, and E, the amount of copper added was reduced to 0.5 p.p.m. All milk was then dried under a vacuum of from 22 to 25 inches. Upon completion of the drying, the powder was ground in an electric food mixer to insure uniformity in particle size.

The following data were obtained on all samples: per cent fat; per cent moisture, peroxide value; solubility, pH of reconstituted sample and flavor of reconstituted sample.

Moisture determinations were made by weighing samples of approximately 1 gram into lead foil dishes with dimension of 5.5 cm. by 1.5 cm. The samples were then heated in the thermostatically controlled vacuum oven of the Mojonnier Tester, under a vacuum of approximately 22 inches, until constant weight was reached. The per cent moisture was then calculated from the loss in weight of the heated samples.

Peroxide values were determined by the method for milk powder as recommended by Smith (15) and the results recorded as M values.<sup>1</sup>

The method of the American Dry Milk Institute (18) was used in determining the solubility index of all powders.

Flavor observations were made on all samples for the degree of tallowiness after reconstitution. The judging was done by the authors assisted by W. J. Corbett. The samples were reconstituted as follows:

Ten grams of the dry milk powder were added to 80 ml. of distilled water which had previously been warmed to 100° F. agitated in a malted milk mixer for 30 seconds then cooled.

<sup>1</sup> M = Millimoles of peroxide per kilogram of fat.

The pH determinations were made on the same sample as was used for the flavor observations.

#### KEY TO SAMPLES

All samples are designated in the following manner:

Capital letters A, B, C, etc. indicate the experiment.

The various antioxidants are indicated by small numbers 1, 2, 3, etc. and are as follows: 1—Butyl ester of the amino acid tyrosine; 2—Hydroquinone; 3—Gum guaiac; 4—Avenex; 5—Enzylac; 6—Ascorbic acid; 7—Sodium citrate; 8—*S. lactis* starter; 9—Control.

Numbers accompanied by a small c such as 1c, 2c, 3c, etc. indicate an addition of copper to the milk before drying.

Different types of containers are designated as follows: p—plain cardboard; pp—paraffined cardboard; ap—Avenized paper bag; t—plain tin container (friction cap); et—sanitary enameled (lacquered) tin container (sealed).

#### EXPERIMENTAL RESULTS

##### *Use of Antioxidants*

Raw milk with an acidity of 0.17 per cent was processed in accordance with the general plan and condensed to a ratio of 3.59:1.

The weights of the various antioxidants were figured on the basis of 4 per cent milk and the following concentrations were made:

1. Butyl ester of tyrosine	0.03%
2. Hydroquinone*	10 ppm
3. Gum guaiac	5 ppm (dissolved in 5 cc. of ethyl alcohol)
4. Avenex (#9)	0.25%
5. Enzylac	1 part per 25,000 parts of milk
6. Ascorbic acid	0.01%
7. Sodium citrate	0.20%
8. <i>S. lactis</i> starter	1 ml per pound
9. Control	No additions

With the exception of the Avenex, Enzylac and *S. lactis* starter, all antioxidants were added at the time of drying. In the case of these products the treated milks were stored at 40° F. for 6–8 hours before drying.

The required weight of Avenex flour was made into a gruel with 50 ml.

\* Although a patent has been granted Nitardy (Nitardy, F. W., U. S. Patent No. 1,879,762, Sept. 27, 1932) for the use of hydroquinone as an antioxidant in fat soluble vitamin concentration, the American Medical Association has not approved its use in this manner because of its toxic effect. (Jour. Amer. Med. Assoc., 109: 1454. 1937). The legality of adding any antioxidant material to milk before drying would need to be established before its use could be recommended. The authors' interest in the products studied was mainly scientific, though the practicable possibilities of a study of this nature must always be recognized.

of hot H<sub>2</sub>O and then added to the milk. The bottom portion containing the Avenex residue was not included with the milk that was dried.

The Enzylac powder was added to the previously pasteurized and condensed milk at a temperature of from 95°–100° F. The milk was then heated to 150° F. as rapidly as possible and held at that temperature for 30 minutes to inactivate the enzyme.

The *S. lactis* starter was added at the rate of 1 ml. per pound of milk which was then held at 70° F. until an increase of 0.02 per cent in titratable acidity was obtained. Copper sulphate was added to half of each sample as explained under experimental procedure. Sixty grams of each lot of powder, with and without copper, was then packed in a 4 oz. brown glass bottle and sealed with a screw cap. No attempt was made to regulate the moisture content of the powders. All samples were stored at room temperature (thermostatically controlled at 72° F.) and were examined at regular intervals. The fat content of a composite sample of the powders in this experiment tested 29.31 per cent.

TABLE 1

*Degree of tallowy flavor in relation to various antioxidants*

Sample	Number of days in storage at room temperature					
	45	72	101	134	191	231
Degree of tallowy flavor*						
A 1		1	2	1	3	5
1c	2½	7	5	6	6	7
2		1		1	3	3
2c	1	2½	2	3	2½	5
3	**					
3c	½	2	3	3	3	6
4		2	3	2	4	3
4c	3	8	8	9	8	8
5	rancid	rancid	rancid	rancid	rancid	rancid
5c	1	6	5	9	7	7
6		½	1	1	3	3½
6c	1½	4	2½	3	4	5
7	***	½		1	2	4
7c	3	3	4	5	5	7
8		½	3	1	2	4
8c	5	8	10	10	7	7
9	1	½	2	2	3	4½
9c	3	4	5	6	7	7

\* The numbers 1, 2, 3, etc., indicate an increasing degree of tallowy flavor.

\*\* All gave slight gum flavor.

\*\*\* Slightly salty.

The data in table 1 and figures 1–8 offer evidence that the development of the tallowy flavor in powdered whole milk can be retarded and prevented by the addition of antioxidants. Of the various antioxidants used gum guaiac was the most effective (fig. 3). When this compound was present in powders containing no additions of copper, the powder was still free from tallowy flavor after storage for 231 days at room temperature, while the con-

trol had developed the flavor defect by the end of 45 days. With the addition of 2 ppm of copper, the powder developed the tallowy flavor after 45 days but the degree of oxidized flavor in the control samples was much worse with the same period of storage. The intensity of the off-flavor in the control samples increased rapidly, while paired samples containing gum guaiac showed a much slower rate of the oxidized flavor development.

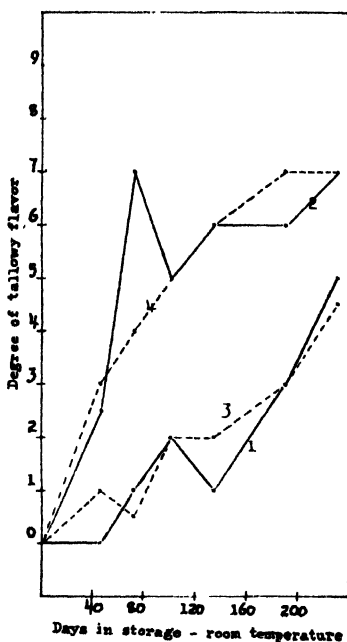


FIG. 1. Effect of the butyl ester of tyrosine on tallowy flavor development.

Legend to curves:

1. Butyl ester of tyrosine
2. Butyl ester of tyrosine — with added copper
3. Control
4. Control — with added copper

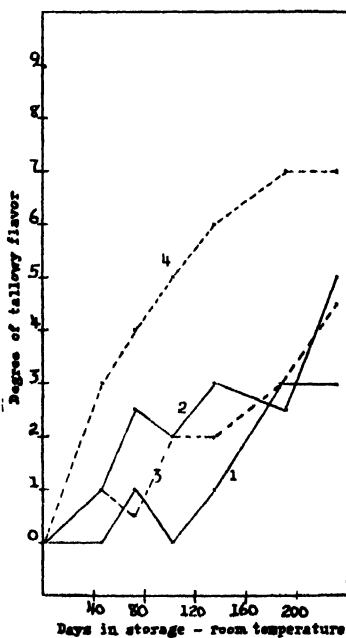


FIG. 2. Effect of hydroquinone on tallowy flavor development.

Legend to curves:

1. Hydroquinone
2. Hydroquinone -- with added copper
3. Control
4. Control — with added copper

Hydroquinone, ascorbic acid and sodium citrate (figs. 2, 6 and 7) were also effective in retarding the development of the tallowy flavor in powders with and without copper.

Avenex did not prevent or retard the flavor defect in samples containing copper although the Avenex samples which did not contain copper were free from tallowy flavor for the first 45 days, while the control sample had a noticeable off flavor.

Powder without added copper prepared from milk inoculated with small amounts of *S. lactis* starter, developed tallowy flavor at a slower rate than

did the control, but samples containing copper became tallowy at a more rapid rate than the control sample to which copper had been added.

Powder made from milk to which Enzylac (fig. 5) had been added before drying developed a disagreeable rancid flavor. Samples that were extremely rancid developed no detectable tallowiness; however, when there was only a slight amount of rancidity tallowiness was evident. Samples containing copper were more oxidized in flavor than the control samples. It is inter-

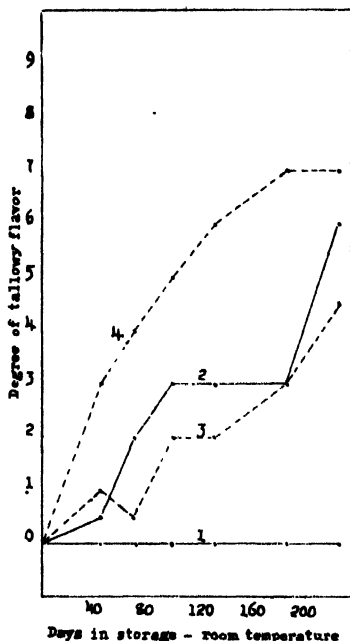


FIG. 3. Effect of gum guaiac on tallowy flavor development.

Legend to curves:

1. Gum guaiac
2. Gum guaiac — with added copper
3. Control
4. Control—with added copper

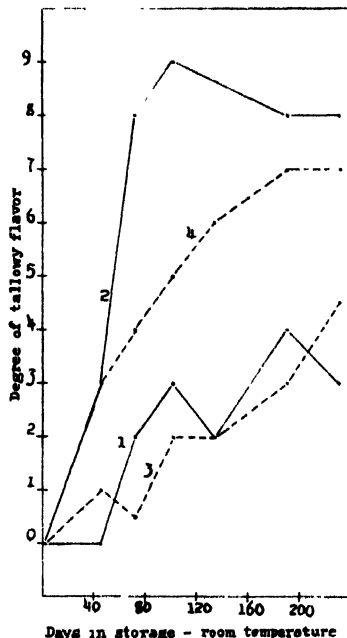


FIG. 4. Effect of avenex on tallowy flavor development.

Legend to curves:

1. Avenex
2. Avenex—with added copper
3. Control
4. Control—with added copper

esting to note that the more tallowy the flavor the less tendency there was for rancidity to develop and vice versa.

It will be noted that there were some instances of slight decreases in flavor intensity which occurred in some samples from one period of examination to the next. Such discrepancies are to be expected, however, because of the chances for error that may be encountered in obtaining data based upon flavor ratings made at different time intervals. The olfactory sense was used in detecting the tallowing flavors and this makes it difficult to

obtain consistent results on the intensity of the flavor defect from time to time.

Moisture determinations were made on all powder samples but no definite correlation was obtained between the percentage of moisture and the degree of tallowy flavor. The moisture content of the powders probably was not high enough in any of the samples to hasten the flavor deterioration. This is in keeping with the results obtained by other investigators who have found that moisture is not a factor in the deterioration of milk powders until the amount present exceeds 5 per cent.

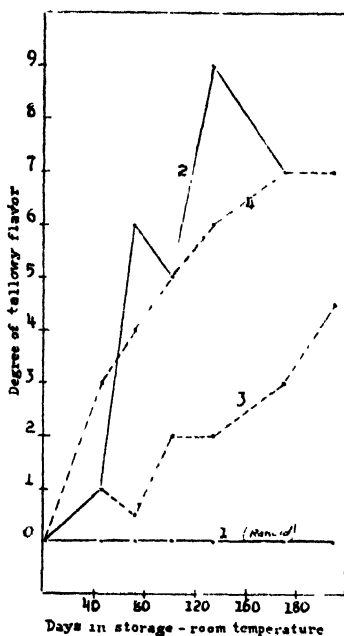


FIG. 5. Effect of Enzylac on tallowy flavor development.

Legend to curves:

1. Enzylac
2. Enzylac—with added copper
3. Control
4. Control—with added copper

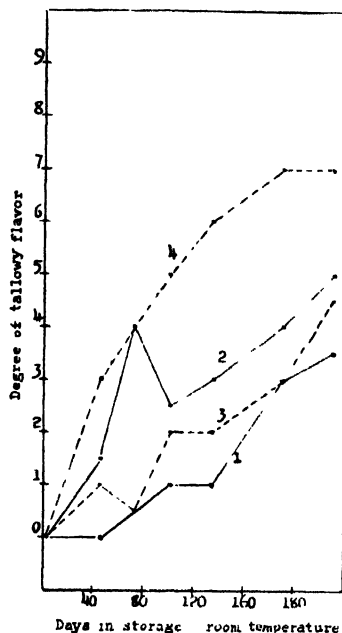


FIG. 6. Effect of ascorbic acid on tallowy flavor development.

Legend to curves:

1. Ascorbic acid
2. Ascorbic acid — with added copper
3. Control
4. Control—with added copper

A slight decrease in moisture content was noted after the first month of storage. This is likely due to the moisture content of the powders not having reached equilibrium at the time the powders were placed in the glass sample bottles. After that initial change the other differences in moisture value were in most cases within the range of experimental error for this type of determination. Under the conditions of this experiment where the

moisture content of the powders varied from a low of 2.32 to a high of 6.64 per cent and the temperature of storage remained constant at approximately 72° F., there was, in general, no significant effect of any of the variables studied on the solubility of milk powders. It should be noted, however, that in the case of samples containing sodium citrate, there is marked evidence that the presence of this salt causes an increase in solubility of the milk powder.

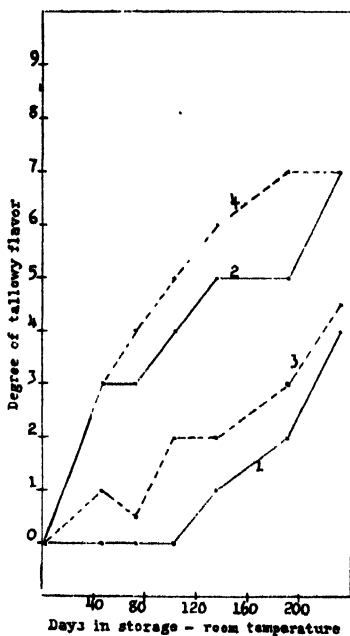


FIG. 7. Effect of sodium citrate on tallowy flavor development.

Legend to curves:

1. Sodium citrate
2. Sodium citrate—with added copper
3. Control
4. Control—with added copper

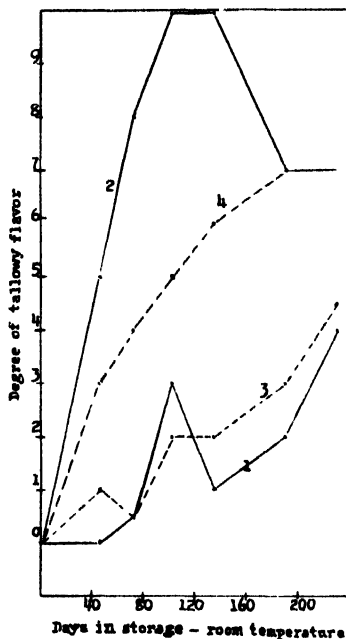


FIG. 8. Effect of bacterial culture on tallowy flavor development.

Legend to curves:

1. *S. lactis* starter
2. *S. lactis* starter—with added copper
3. Control
4. Control—with added copper

Peroxide values have been used with some success in determining the extent of oxidation of fats and oils and it was thought that perhaps it might be possible to follow the oxidation of fat in the milk powders by a chemical as well as an organoleptic method. It will be noted from figures 9, 10, 11, and 12 (plotted from data in tables 1 and 2) that no samples gave a peroxide value until after 40 days of storage. It should also be noted that at the end of 45 days only one sample gave a degree of oxidized flavor of more than 5. Further, after 72 days peroxide values were obtained for 4 of the 6 samples that had a tallowy flavor intensity of 5 or more. In gen-

eral, the tendency was to obtain a peroxide value when the degree of tallowiness in the samples was 5 or greater. At the end of 185 days of storage, two samples which had previously given peroxide values (samples 1c and 6c) no longer did so. Also the peroxide values had increased in some samples and decreased in others. One fact that can be noted is that the

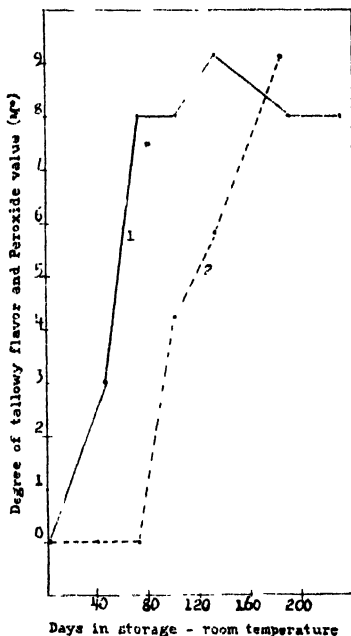


FIG. 9. Correlation between degree of tallowy development and peroxide value. (Avenex with added copper.)

Legend to curves:

1. Degree of tallowy flavor
2. Peroxide value (M) see experimental procedure

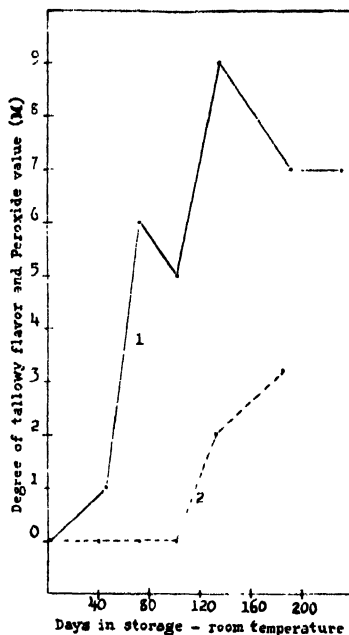


FIG. 10. Correlation between degree of tallowy development and peroxide value. (Enzylac with added copper.)

Legend to curves:

1. Degree of tallowy flavor
2. Peroxide value (M)

tendency previously noted for peroxide values to be obtained only on samples rating 5 or higher in flavor score is not confirmed, as no samples without copper gave a degree of tallowiness of more than 5. It is evident from these data that the olfactory method of detecting tallowiness is more sensitive than the chemical method used in this experiment. The results obtained indicate that peroxide values are not suitable for detection of early oxidation but the accuracy may increase as the oxidation proceeds.

Hydrogen ion determinations made on the reconstituted milk indicated that all samples fell within the range of normal fresh milk (pH 6.4–6.8). There was no definite tendency for changes in pH during storage. Those



TABLE 2

*Effect of storage at room temperature upon peroxide values*

Sample	Days in storage					
	Fresh	40	72	101	132	185
M* Values						
A 1	.. **					
1c			5.027	0.215	0.319	
2						
2c						
3						
3c						0.665
4						
4c				4.232	5.826	9.141
5						0.229
5c					2.029	3.266
6						
6c			0.575			
7						0.292
7c			1.149	1.392		5.605
8						
8c			2.384	8.035	32.196	7.635
9						0.231
9c					0.813	1.955

\*  $M = \frac{T \times N \times 500}{W}$      $W = \text{gm. of fat.}$      $T = \text{ml. of sodium thiosulfate.}$

\*\* No values found.

samples that were treated with Enzylac were rancid (pH 6.60–6.65) indicating hydrolysis of the fat and the presence of free fatty acids. The addition of sodium citrate increased the pH to the upper limit (pH 6.8) of normal milk. These results indicate that reconstituted milk made from powder stored for several weeks does not differ much if any from normal milk in pH. It is also evident that the buffer capacity of the milk was not materially affected by the drying or any of the variables studied by this experiment.

#### *Effect of Pasteurizing Temperature*

In order to determine the effect of pasteurizing temperature on the keeping quality and physical properties of milk powder, raw milk with 0.165 per cent acidity was processed in the regular manner except that pasteurizing temperatures of 150° F., 170° F. and 190° F. for 30 minutes were used. The lots pasteurized at 170° F. and 190° F. for 30 minutes were condensed in laboratory condensing equipment and that pasteurized at 150° F. in the creamery vacuum pan. At the time of drying, copper at the rate of 0.5 ppm was added to one-half of each lot of milk.

No attempt was made to regulate the moisture content of the powder and all samples were packed in brown glass bottles with screw caps and stored at room temperature.

The fat content of the powders in this experiment was as follows:

Pasteurized at 150° F.	30.31%
" " 170° F.	30.85%
" " 190° F.	30.53%

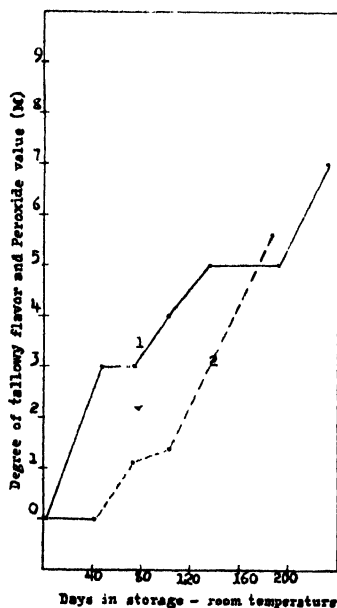


FIG. 11. Correlation between degree of tallowy flavor and peroxide value. (Sodium citrate with added copper.)

Legend to curves:

1. Degree of tallowy flavor
2. Peroxide value (M)

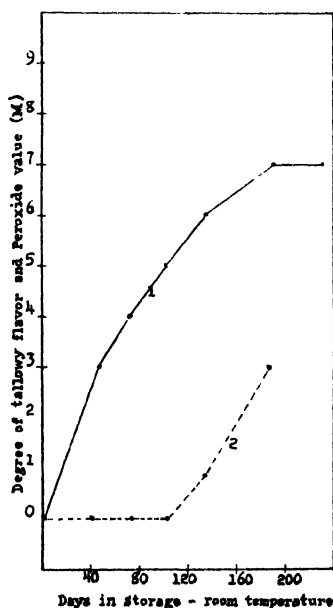


FIG. 12. Correlation between degree of tallowy flavor and peroxide value. (Control with added copper.)

Legend to curves:

1. Degree of tallowy flavor
2. Peroxide value (M)

TABLE 3

*Relation of pasteurization temperature to the storage properties of milk powder*

Sample	Degree of tallowy flavor Days in storage			ml. of insoluble material Room temperature		
	Fresh	26	67	Fresh	26	27
B 150° F. . . .	..	1½	5	2.00	2.10	3.70
150° F. with Cu.	..	4	8	2.10	2.20	3.60
170° F. . . . .	..	2½	1	2.80	3.00	3.50
170° F. with Cu. .	..	3	3½	3.10	3.40	3.80
190° F. . . . .	..	3½	6	2.80	3.40	5.60
190° F. with Cu.	..	4½	9*	2.80	4.50	8.00

\* Defect not typical tallowy but more of a scorched flavor due to browning of powder.

The data in table 3 indicate that the temperature at which the milk is heated before drying is related to the keeping quality of the powder. Best results were obtained when the milk was heated to 170° F. for 30 minutes. It is also evident that the powder made from milk pasteurized at 150° F. for 30 minutes had a better flavor after storage than that made from milk heated at 190° F. for 30 minutes.

Figure 13 graphically presents the data in table 3 and clearly pictures

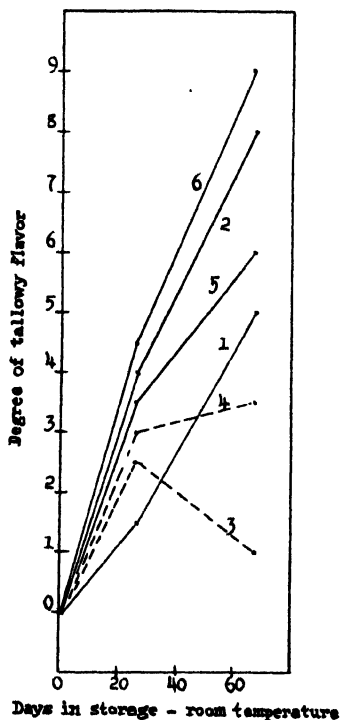


FIG. 13. Relation of pasteurizing temperature to tallowy flavor development.

Curve Number:

1. Pasteurized at 150° F.
2. " " 150° F.
3. " " 170° F.
4. " " 170° F.
5. " " 190° F.
6. " " 190° F.

Numbers 2, 4 and 6 with added copper.

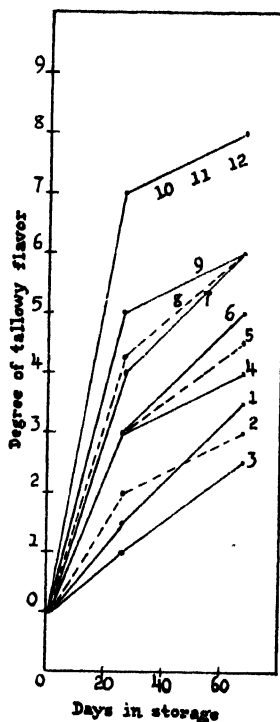


FIG. 14. Relation of moisture content to tallowy flavor development.

Curve No.	Per cent moisture when stored	Storage temperature
1.	2.32	0° C.
2.	3.44	0° C.
3.	5.39	0° C.
4.	2.32	15° C.
5.	3.44	15° C.
6.	5.39	15° C.
7.	2.32	20° C.
8.	3.44	20° C.
9.	5.39	20° C.
10.	2.32	37° C.
11.	3.44	37° C.
12.	5.39	37° C.

the superior keeping quality of the powder made from milk heated at 170° F. for 30 minutes.

The comparison of the solubility indices of powders made from milk heated to the 3 different temperatures shows that the least soluble powders were those made from milk heated to 190° F. The close correlation in flavor between powders made from milk heated to 150° F. and 170° F. and the lack of correlation in the solubility of these two sets of powders indicates that there is no relation between solubility index and flavor change.

### *Effect of Moisture Content*

The milk used in this experiment was processed in the regular manner. At the time of drying 0.5 ppm of copper were added to one-half of each lot of milk. The moisture content of one set of samples was adjusted to approximately 5 per cent by spreading the powder out on parchment paper in a humid room until the desired moisture content was reached. As a control, the powder as it came from the drier with a moisture content of approximately 3.5 per cent was used. By circulating 100° F. air over powder spread on parchment paper a product with a moisture content of 2 per cent was obtained. The fat content was 28.44 per cent in the control sample. All samples were placed in brown glass bottles with screw caps.

In a second experiment a series of 5 samples was prepared in which the moisture contents were varied from 2 to 5 per cent at 0.75 per cent intervals. This was accomplished by adjusting two lots of powder to a moisture content of 2 per cent and 5 per cent respectively and mixing the proper proportions of these high and low moisture powders to obtain the intermediate samples.

Each series of samples was stored at the following temperatures: 0° C., 15° C., 20° C., and 37° C. No copper additions were made to any of the samples and the fat content of the powder before adjusting the moisture was 30.34 per cent.

TABLE 4

*Relation of variation of moisture content to the storage properties of milk powder*

Sample	Degree of tallowy flavor after indicated number of days in storage at room temperature		
	Fresh	54	116
C 1*		1	8
1 with Cu.		4½	9**
C m		2	4
m with Cu.		5	4
C h		3	7
h with Cu. . .		8**	10**

\* 1 low moisture content powder.

m medium moisture content powder.

h high moisture content powder.

\*\* Defect not typical tallowy but more of a scorched flavor due to browning of powder.

It is recognized that the procedure followed in this experiment is open to criticism. In the first place there is some question as to whether or not the adjustments in moisture content were made uniformly throughout each separate lot of powders; it is also a question as to whether or not the treatment to which the powder was subjected in order to adjust the moisture content was related in some way to the changes in the appearance and flavor of the powders that occurred during storage.

TABLE 5

*Relation of variation in moisture content and storage temperature to the storage properties of milk powder*

Sample	Per cent moisture when placed in storage	Degree of tallowy flavor after indicated days in storage			ml. insoluble material days in storage		
		Fresh	26	67	Fresh	26	67
0° C.	2.32		1½	3½	1.30	1.40	1.50
	3.14		2	3½	1.40	1.40	1.60
	3.44		2	3	1.40	1.30	1.50
	4.64		1	3	1.60	1.50	1.60
	5.39		1	3	1.90	2.00	1.70
15° C.	2.32		3	4	1.30	1.30	1.80
	3.14		3	4	1.40	1.40	1.70
	3.44		3	4½	1.40	1.30	1.60
	4.64		3	5	1.60	1.45	1.30
	5.39		3	5	1.90	1.90	1.50
20° C.	2.32		4	6	1.30	1.20	1.70
	3.14		4½	6½	1.40	1.50	1.60
	3.44		4½	6	1.40	1.30	1.70
	4.64		4½	6	1.60	1.30	2.10
	5.39		5	6	1.90	2.50	2.10
37° C.	2.32		7	8*	1.30	1.60	2.00
	3.14		7	8*	1.40	3.00	3.90
	3.44		7	8*	1.40	2.60	2.80
	4.64		7	8*	1.60	2.60	5.90
	5.39		7	8*	1.90	3.60	9.00

\* Defect not typical tallowy but more of a scorched flavor due to browning of powder and increasing in intensity with increase in moisture content.

It is safe to conclude, however, from the data (table 5) that the temperature at which the powder is stored is much more of a factor as far as stability or flavor is concerned than is the moisture content. It should be observed that all samples stored at 37° C. in the experiment recorded in table 5 and those samples of the highest moisture content recorded in table 4 turned brown and developed a caramel flavor during the storage. At the higher storage temperature (37° C.) all samples turned brown regardless of moisture content. It is evident that there is a critical point in the neighborhood of 5 per cent moisture which represents the upper limit of moisture for samples to be stored at room temperature without deterioration in

flavor and color. At the higher storage temperatures (20° C. or above) discoloration will take place regardless of the moisture content. This change in the color of powdered milk as related to storage temperature is likely a phenomenon of the same order as occurs in sweetened condensed milk when stored at 20° C. or above.

It should also be observed that those samples which discolored during storage became less soluble indicating a change in the protein constituents of the powder. It is also true that these same samples had a lower pH value than the ones which showed no change in color during storage.

### *Effect of the Type of Container*

The effect of containers on the keeping quality of the powder was investigated by packing the powder in the following types of containers and storing them at room temperature: p—plain cardboard cup and cover; pp—

TABLE 6  
*Relation of the type of container to degree of tallowy flavor development*

Sample	Days in storage*		Sample	Days in storage*		Sample	Days in storage*	
	35	206		35	206		35	206
D 1 p	?	2½	D 4 p		3½	D 7 p	½	2
pp	?	4	pp		5	pp	½	1
ap		2	ap		2½	ap		2
t	?	5	t		6	t		4
et	?	1½	et		4	et		
D 1c p	3½	8	D 4c p	3	8	D 7c p	5	8
pp	3	5	pp	2	8	pp	6	7
ap	3	8	ap	2	7	ap	3½	9
t	3	10	t	3½	9	t	5	10
et	3	3½	et	2	5	et	6	7
D 2 p	½	2½	D 5 p	Sl.R.**	3	D 8 p	½	4
pp	½	3	pp	Sl.R.	2	pp	½	5
ap	½	2	ap	Sl.R.	2	ap	½	6
t	½	4	t	Sl.R.	5	t	1	7
et	½	1½	et	Sl.R.	1½	et	½	3
D 2c p	4	4	D 5c p	Vs.R.***	10	D 8c p	6	10
pp	4	2½	pp	Vs.R.	10	pp	7	11
ap	4	3½	ap	Vs.R.	9	ap	5	10
t	5	7	t	Vs.R.	8	t	7	13
et	4	5	et	Vs.R.	6	et	7	13
D 3 p		1	D 6 p		4	D 9 p	½	5
pp		½	pp	1	3	pp		4
ap		½	ap		5	ap		4½
t		1	t	1	7	t	1	3
et		½	et		3	et		6
D 3c p	2	8½	D 6c p	5	11	D 9c p	4	9
pp	2	2	pp	7	8	pp	4	7½
ap	2	8	ap	4	5	ap	3	8
t	2	2	t	6	13	t	4½	9
et	2	1	et	5	13	et	4	7

\* Stored at room temperature.

\*\* Sl.R. = slightly rancid.

\*\*\* Vs.R. = very slightly rancid.

plain cardboard cup and cover dipped in melted paraffin and sealed with paraffin; ap—paper coffee bags lined with Avenized paper\*; t—plain tin container with friction type cap; et—sanitary enameled #1 tin (sealed in the regular manner).

The raw milk, with an acidity of 0.15 per cent was processed as indicated under experimental procedure and condensed to a ratio of 2.6–1. Equivalent amounts of the same antioxidants were added at the time of drying and 0.5 ppm of copper was also added to one-half of each lot of milk. No attempt was made to regulate the moisture content of the powder. The average fat content of the powders in this experiment was 27.69 per cent.

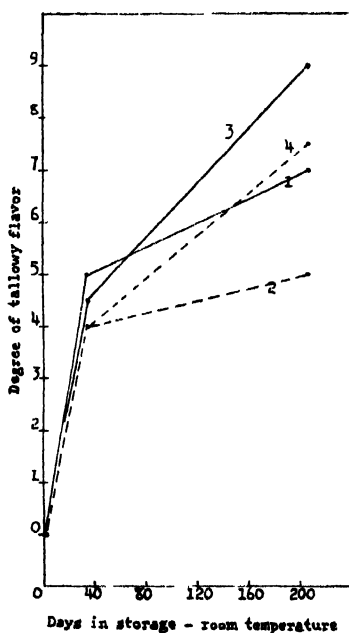


FIG. 15. Correlation between type of container and tallowy flavor development.

Legend to curves:

Hydroquinone with added copper

1. Plain tin

2. Enameled tin

Control with added copper

3. Plain tin

4. Enameled tin

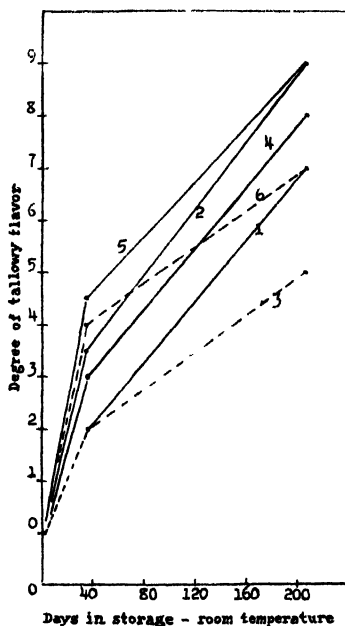


FIG. 16. Correlation between type of container and tallowy flavor development.

Legend to curves:

Avenex with added copper

1. Avenized coffee bag

2. Plain tin

3. Enameled tin

Control with added copper

4. Avenized coffee bag

5. Plain tin

6. Enameled tin

It has been known for a number of years that protection from air and light will improve the keeping quality of powdered milk. The data in table 6 further substantiate the work of other investigators in this respect. When

\* Courtesy of Musher Foundation.

the powders were stored in plain cardboard containers, tallowy flavor developed rapidly in all cases.

The treatment of wrappers with oat flour (Avenex) has been shown to improve the keeping quality of butter and, therefore, the effect of this type of package was studied in connection with milk. To a certain extent the Avenized bags protected the powder from oxidation. It is also apparent that this type of package resulted in a fresher and better flavored product in all samples during the first 40 days of storage. This fact is not clearly shown in the data, but was noticeable when the samples were judged.

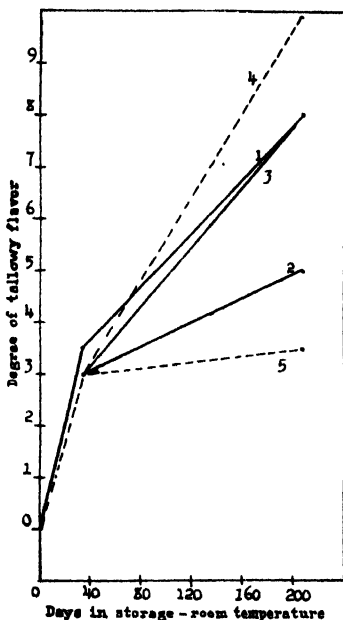


FIG. 17. Correlation between type of container and tallowy flavor development.

Legend to curves:

Butyl ester of tyrosine with added copper.

1. Paper
2. Paraffined paper
3. Avenized coffee bag
4. Plain tin
5. Enameled tin

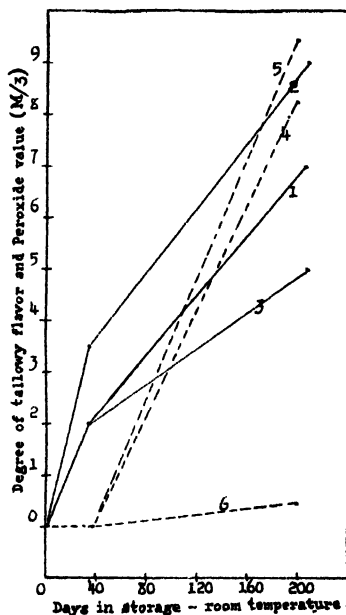


FIG. 18. Correlation between type of container and tallowy flavor development.

Legend to curves:

Control with added copper

1. Paper
2. Paraffined paper
3. Avenized coffee bag
4. Plain tin
5. Enameled tin

Data from table 6 shown graphically in figures 15, 16, 17, and 18, indicate that the type of container used has a definite relationship to the development of tallowy flavor. The plain tin containers, friction sealed, were less satisfactory than the enameled tin container. A heavy coating of paraffin on cardboard and sealing with paraffin gave protection with some samples but the results were not as consistent as in the case of the enameled tin.



The moisture content of the powders varied with the type of container and changed considerably during storage with all containers except in the case of the enameled tin. There was a slight variation in moisture content of the powder stored in the enameled tin container. However, this variation was well within the range of experimental error. In general, the containers sealed against air penetration (paraffined paper and the sanitary container) protected the powders to the best advantage as far as a change in moisture content was concerned.

The effect of the various antioxidants was again shown in this series of samples. Gum guaiac, hydroquinone, ascorbic acid, butyl ester of tyrosine, sodium citrate and Avenex were effective in the order named in preventing the development of a tallowy flavor.

TABLE 7  
*Relation of the type of container to peroxide values*

M* Values					
Sample	Days in storage**		Sample	Days in storage**	
	37	199		37	199
D 1 p	***		D 4 p		
pp			pp		
ap			ap		
t			t		
et			et		
D 1c p		16.71	D 4c p	23.48	
pp			pp	3.24	
ap		14.89	ap	24.83	
t		9.33	t	28.37	
et		6.68	et	1.36	
D 2 p			D 5 p		
pp			pp		
ap			ap		
t			t		
et			et		
D 2c p			D 5c p	20.01	
pp			pp	5.04	
ap			ap	26.05	
t			t	1.18	
et			et	4.34	
D 3 p			D 6 p		
pp			pp		
ap			ap		
t			t		
et			et		
D 3c p		3.57	D 6c p	20.18	
pp		0.71	pp	64.29	
ap		3.32	ap	18.58	
t		0.19	t	40.12	
et		0.53	et	60.68	
			D 7 p		
			pp		
			ap		
			t		
			et		
			D 7c p		3.29
			pp		9.38
			ap		32.57
			t		14.80
			et		10.76
			D 8 p		
			pp		
			ap		
			t		
			et		
			D 8c p		26.55
			pp		55.66
			ap		5.53
			t		16.58
			et		
			D 9 p		
			pp		
			ap		
			t		
			et		
			D 9c p		20.40
			pp		3.95
			ap		25.15
			t		3.18
			et		37.50

\* Values see experimental procedure.

\*\* Stored at room temperature.

\*\*\* ..... = No values found.

Data from tables 6 and 7 indicate that there is some correlation between the degree of tallowness, peroxide values and type of container. Enameled tin containers retarded the development of peroxides as well as the tallowy flavor. Avenized bags were more effective in this respect than the plain tin containers but not as effective as the enameled tin cans.

The solubility of the milk powder was not affected by the type of container used.

### *Effect of Storage Temperature*

For the study of the effect of storage temperature a series of three sets of samples were prepared.

1. The raw milk was processed in the regular manner except that it was pasteurized at 170° F. for 30 minutes. At the time of drying, copper at the rate of 0.5 ppm was added to one-half of the lot of milk and the powder samples were packed in brown glass bottles with screw caps and stored at 0° C. and 42° C. The fat content of the powder was 30.85 per cent.

2. The raw milk was processed in the regular manner and copper at

TABLE 8

*Relation of storage temperature to the development of tallowy flavor*

Sample	Samples stored in Avenized bags				Samples stored in plain tin containers			
	Storage temperature				Storage temperature			
	0° C.		42° C.		0° C.		42° C.	
	Days in storage		Days in storage		Days in storage		Days in storage	
	62	117	49	117	62	117	49	117
Degree of tallowy flavor					Degree of tallowy flavor			
E 1	heated			2½	heated	1	3	3
1c	2	2	4	5	1	4	burnt	9
2	heated		2	5	heated	1	burnt	8
2c	2	1	1	4½	heated	3½	1	4
3						1		
3c		½				3		
4	heated		2	1½		3	1½	3½
4c		5	3	5	2	5	3	4½
5	rancid	rancid	rancid	rancid	{ rancid	1	{ rancid	rancid
5c	rancid	1	rancid	1	{ heated		{ burnt	
					rancid	3	{ rancid	rancid
							{ burnt	
6			1	1½		1	1	3
6c	heated	1	2	6	1½	3	burnt	9
7			1	1½	heated	2	1	2½
7c		7	6	6		5	3	4½
9			heated	1½	heated	2	3	3½
9c	heated	5	5	7	½	5	5	5

the rate of 0.5 ppm was added at the time of drying. The moisture content of the powder was adjusted to approximately 5 per cent by spreading on parchment paper in a humid room until sufficient moisture had been absorbed. The samples were packed in brown glass bottles with screw caps and stored at 0° C. and 42° C. The fat content of the powder before adjusting the moisture was 28.44 per cent.

3. The same processing procedure was used as under experiment A and

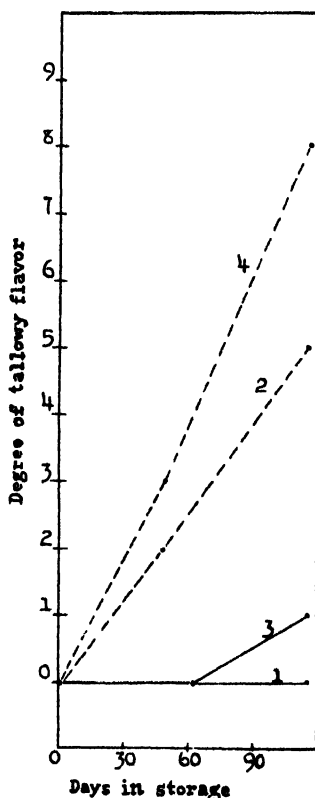


FIG. 19. Relation of storage temperature to degree to tallowy flavor development.  
Curve No.

Hydroquinone	Storage Temperature	Type of Container
1. . . . .	0° C.	Avenized coffee bag
2. . . . .	42° C.	Avenized coffee bag
3. . . . .	0° C.	Plain tin
4. . . . .	42° C.	Plain tin

the same antioxidants, in equivalent amounts, were added. Copper at the rate of 0.5 ppm was added to one-half of the milk before drying. All samples were packed in the following containers: 1. paper bags lined with Avenized paper; 2. plain tin containers with a friction top. They were stored at 0° C. and 42° C. The fat content of the powder was 30.85 per cent.

The data in table 8 indicate that the storage temperature is important in the rate of tallowy flavor development. All samples stored at 0° C. in Avenized bags had developed little or no tallowy flavor at the end of 117 days of storage. Similar samples stored at 42° C. were definitely tallowy and some were slightly discolored at the end of an equal length of time. Samples stored in plain tin (with friction top) deteriorated more rapidly at 0° C. than did the samples kept in Avenized bags at the same temperature. Storage at 42° C. in plain tin resulted in an increase in the susceptibility toward oxidation and some of the samples had a caramelized flavor and were discolored. A typical example is graphically illustrated in figure 19.

The moisture content of the samples in Avenized bags stored at 0° C. increased as much as 6 per cent because of the high humidity in the refrigerated room.

The moisture content of the powder samples held at 0° C. in tins, with the friction seal, remained nearly constant during storage. Samples in the same type of containers stored at 42° C. increased slightly in moisture content during storage. This change in moisture content may have been a result of the inter-reaction between the lactose and casein at this high temperature. This again may account for the fact that the powders that de-

TABLE 9  
*Relation of storage temperature to peroxide values\**

Sample	Samples stored in Avenized bags				Samples stored in plain tin containers			
	Storage temperature				Storage temperature			
	0° C.		42° C.		0° C.		42° C.	
	Days stored		Days stored		Days stored		Days stored	
	47	121	47	121	47	121	47	121
E 1	**							
1c								2.111
2								0.691
2c								
3								
3c								
4								
4c				0.915		0.704		
5								
5c								2.111
6								
6c								2.601
7								
7c								
9								
9c				1.898		0.591		

\* M values—see experimental procedure.

\*\* No values found.

creased in solubility during storage at 42° C. showed a tendency towards an increase in moisture content.

Even though the powder in the Avenized bags increased in moisture, when stored at 0° C. and 42° C., there was very little change in solubility. The samples packed in tin, however, showed a greater fluctuation in solubility when stored at 42° C. than did the samples in the paper container. A marked decrease in solubility took place in the powder when stored in tin at 42° C., although this type of container was more or less air tight as indicated by the constant moisture values.

As previously noted there was a decrease of approximately 4-1 in solubility index of the samples to which sodium citrate was added before drying, showing a marked increase in solubility when this salt is present.

TABLE 10  
*Relation of storage temperature and moisture content to the storage properties of milk powder*

Sample		Per cent moisture Days in storage			Flavor Days in storage			ml. insoluble Days in storage		
Temp.	Number	Fresh	57	97	Fresh	57	97	Fresh	57	97
0° C.	Ch*	5.46	3.46	3.31		4	6	2.40	1.80	1.50
	Chc	5.20	5.03	5.08		6**	8**	3.50	8.00	10.00
42° C.	Ch	5.46	2.74	3.00		7	9**	2.40	7.20	9.00
	Chc	5.20	5.00	4.46		8**	11**	3.50	12.50	12.00

\* h High moisture powder.

\*\* Defect not typical tallowy but more of a scorched flavor due to browning of powder.

Peroxide values (table 9) were found only after 121 days of storage at 42° C. The conclusions, previously drawn from table 6, of the increase in susceptibility of powders to oxidation when stored in plain tin and held at high temperatures are further substantiated by the results of this experiment. Only two samples stored in Avenized paper bags at 42° C. gave slight values while values were found for 4 samples of powder stored in tin at 42° C.

The pH values of the reconstituted milk gave little indication of any definite trend except in the case of those samples with a decreased solubility. As previously shown (Experiment A) a drop in pH occurs when there is a noticeable decrease in solubility and change in the color of the powder.

The data in table 10 further confirm the results indicated in tables 5 and 8 in that there is an increase in rapidity of the development of the tallowy flavor at high storage temperature. This effect is more marked where copper is present.

#### CONCLUSIONS

1. Sufficient evidence has been obtained to indicate that it is possible to retard the development of oxidized flavor in whole milk powders by adding antioxidants to the milk before it is dried.

2. All antioxidants studied were found to have some effect in retarding the development of oxidized flavor in milk powder. These products varied, however, in their effectiveness and may be grouped in this respect as follows:

Group 1. (Most effective antioxidants)

- a. Gum guaiac
- b. Hydroquinone

Group 2 (Intermediate in effectiveness)

- a. Ascorbic acid
- b. Sodium citrate

Group 3 (least effective)

- a. Butyl ester of tyrosine
- b. Avenex
- c. Enzylac
- d. Bacterial culture

3. Powder made from milk heated to 170° F. for 30 minutes is less likely to develop an oxidized flavor than that made from milk heated to a higher (190° F.) or lower (150° F.) temperature.

4. High moisture (5 per cent or above) content in the powder and high (20° C. or above) storage temperatures increase the rate of oxidized flavor development.

5. The conditions found favorable for brown discoloration of the powder during storage were as follows:

- a. Preheating the milk at a high (190° F.) temperature.
- b. A moisture content of 5 per cent or higher.
- c. Presence of added copper salts.
- d. Storing at temperatures of 20° C. or higher.

6. The type of container has a bearing upon changes in color, moisture, solubility and flavor during storage. The container features found to be favorable for maintaining a normal condition of the powder were:

- a. Construction which reduced the amount of air infiltration.
- b. Coating the surface of tin containers with lacquer.
- c. Use of paper bags treated with oat flour (Avenex).

7. Discoloration, reduction in solubility, and a lowering of the pH occur concurrently suggesting a common cause. A reaction between the protein and other constituents in the milk is thought to be involved in much the same manner as in the case of the discoloration and thickening of sweetened condensed milk.

8. Addition of sodium citrate to milk before drying greatly increased the solubility of the milk powder.

9. The peroxide value cannot be used to detect early oxidation of the fat. It may be used, however, to show oxidation of a high degree.

10. The development of oxidized flavor in milk powder can be detected at an earlier stage by the sense of taste than by the use of the peroxide test.

11. The peroxide test could not be used to predict the keeping quality of a fresh sample of powdered whole milk.

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## MICHIGAN INVITES YOU

### TO THE OFFICERS AND MEMBERS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION:

The summer meetings of the American Dairy Science Association, which have become so popular, were originated on the campus of the Michigan State College.

Since this first meeting in 1927, with about seventy-five in attendance, these summer meetings have become an outstanding event with record attendance.

We have been looking forward for several years to your return to our campus.

This college, the Oldest Agricultural College in the World, too, has grown and changed in recent years and we are able to offer excellent accommodations in dormitories, auditoriums, class rooms and other means of making your visit both profitable and pleasing to you.

As a former active member, I am pleased to invite you to visit us in Michigan, the Playground of the Nation and a state famous for its dairy development.

*Signed* ERNEST L. ANTHONY,  
*Dean of Agriculture,*  
*Michigan State College.*

### THIRTY-SEVENTH ANNUAL MEETING, MICHIGAN STATE COLLEGE, EAST LANSING, MICHIGAN, JUNE 22-25, 1942 (TENTATIVE PROGRAM)

#### *Sunday, June 21*

2:00 P.M.—9:00 P.M.—Registration

#### *Monday, June 22*

12:00 M. —Preconvention trip, KVP, Parchment (north edge  
of Kalamazoo, Michigan)

8:00 A.M.—9:00 P.M.—Registration

7:00 P.M.—Committee Meetings or Social Period

#### *Tuesday, June 23*

9:30 A.M.—11:00 A.M.—Opening Session (General program)

11:00 A.M.—12:00 M. —Committee meetings

1:30 P.M.—4:00 P.M.—Section program meetings



- 4:00 P.M.—6:00 P.M.—Tour of dairy barns and pastures  
or New Score Card—Judging dairy products  
8:00 P.M.—Social period

*Wednesday, June 24*

- 9:00 A.M.—11:00 A.M.—Section program meetings  
11:00 A.M.—12:00 M. —Committee meetings  
1:30 P.M.—3:30 P.M.—Section program meetings  
3:30 P.M.—4:30 P.M.—Section business meetings  
8:00 P.M.—Social period

*Thursday, June 25*

- 9:00 A.M.—11:00 A.M.—Section program meetings  
11:00 A.M.—12:00 M. —Committee meetings  
1:00 P.M.—3:00 P.M.—Section meetings  
3:00 P.M.—3:30 P.M.—Section business meetings  
3:45 P.M.—5:00 P.M.—Business (General program)  
6:30 P.M.—Banquet

*Friday, June 26*

- 9:00 A.M. —Post-convention trip, Dearborn, Michigan. Visit  
Greenfield Village and Ford Museum.

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## PURIFICATION OF RENNIN FROM COMMERCIAL RENNIN EXTRACT: PROPERTIES OF PURIFIED PRODUCT\*

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The purity of the enzyme, especially with respect to its contamination with pepsin, is of essential importance in understanding the biochemistry of the coagulation of milk by true rennin. Many investigations have been conducted on this general problem from which erroneous conclusions may have been drawn because of the mixed nature of the enzymes in the rennin preparations used. Several attempts to purify this enzyme have been reported but the properties of the purified products do not show much agreement except a marked increase in milk clotting activity in comparison with the crude starting material.

Fenger (1), starting with dried, defatted, powdered calves' stomach mucosa, obtained a product containing 14.00 per cent N, which precipitated from dilute NaCl solution at pH about 3.5–4.0 and which readily dialyzed through parchment. He regarded the purified rennin as a decomposition product of an acid albumin. Its milk clotting activity at 40° C. in 10 minutes, using sweet, certified milk (1:2,310,000) was 770 times that of the original dried mucosa and its peptic activity (1:600 by the U.S.P. method) only 1.7 times that of the starting material. Lüers and Bader (2), starting with sodium phosphate solution extracts of the lead proteinate obtained from sodium acetate extracts of calves' stomach mucosa, purified the rennin by a series of adsorptions on alumina and kaolin and elutions by phosphate buffer. Milk clotting activity was calculated from viscosity determination made at 35° C. for 40 minute activity time (actual time two minutes) and peptic activity was measured by a turbidometric procedure which is stated to estimate about 0.2 mg. pepsin. The most active rennin preparation contained only 0.687 per cent N and the milk clotting activity of the dry substance was calculated to be 1:16,440,000 parts of a boiled, reconstituted

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milk substrate. This was only 39 times that of the dry lead proteinate starting material. Its peptic activity was still 21 times that of the same starting proteinate complex.

More recently Tauber and Kleiner (3) reported the purification of rennin from fresh calves' stomach mucosa. Their purest product, obtained by precipitation with 50 per cent ethanol at pH 5.4, clotted 4,550,000 parts of a reconstituted skim milk containing added  $\text{CaCl}_2$  in 10 minutes at  $40^\circ\text{C}$ . This represented a 2000 fold concentration compared with the original extract. The product contained 14.4 per cent N, 1.19 per cent S, but no P. It is stated that 25 mg. of the rennin did not produce any increase in formal titration of an aqueous suspension of 250 mg. of coagulated egg albumin in three hours at  $40^\circ\text{C}$ . and only a negligible amount of N not precipitable by trichloroacetic acid. No data are actually given on the peptic activity either of the starting material or the purified rennin. Tauber and Kleiner (4) later found that their purified rennin could be rendered inactive by crystalline pepsin. However, Holter (5) has pointed out that the sensitivity of the methods used by these workers for measuring peptic activity is not sufficiently great to justify their conclusion that their purest rennin was devoid of pepsin.

Recently Rao, Rao *et al.* (6) reported that nitrogen is not an integral part of the rennin molecule, seemingly in support of the earlier work of Lüers and Bader. Like in the work of the latter, adsorption on alumina and kaolin were used; also extraction, centrifugation and filtration procedures. However, Schöberl and Rambacher (7) found that rennin eluted from alumina, gives the biuret and diazo reactions.

The purified rennin obtained both by Fenger and by Tauber and Kleiner had the properties of protein. This is in keeping with the widely accepted view that the protein attacking enzymes of the digestive tract are themselves either proteins or perform their function as a part of protein structures. The products obtained by the workers mentioned may also be regarded as somewhat comparable in milk coagulating activity under like conditions. What seems contradictory is the fact that the Tauber-Kleiner rennin, although much less contaminated with pepsin, nevertheless cannot be regarded as showing as great an increase in coagulating activity as obtained by Fenger, on the basis of the probable dry substance contained in the original extract (which unfortunately is not given); it is the extract, not its dry substance, with which Tauber and Kleiner compare their pure rennin.

The recent successful attempts to bring the digestive enzymes into a highly purified state as crystalline proteins prompted us to examine the possibility of applying similar procedures to rennet extract. Although we were not successful in doing this in the present study, nevertheless a high degree of purity was attained. Since rennin and pepsin are the only known milk clotting enzymes in these stomach tissue extracts it seemed justified to

employ the relative milk clotting and peptic activity as a measure of purity. However, we used a much more sensitive, as well as more accurate measure of peptic activity than was used in previous studies of this problem. The method devised is a technical modification of that reported by Anson (8) and is described below. Moreover, we were fortunate in having for starting material a commercial rennet extract,<sup>1</sup> the dry, salt-free material of which apparently already possessed about 3.5 times the milk coagulating activity of the pure rennin of Tauber and Kleiner, as judged by comparison of our data with theirs.

#### RENNIN PURIFICATION PROCEDURE

Considerable preliminary work was first carried out in which determinations were made of the activity of the precipitates and the supernatant liquids, at varying pH, salt concentrations, kinds of salt and after electro-dialysis. The most active rennin was prepared by the following procedure:

Two liters of the rennet extract, a reddish brown liquid, were adjusted to pH 4.5 with 7.0 ml. of concentrated HCl solution. A precipitate formed at once. The mixture was centrifuged for 20 minutes at 2000 R.P.M. The supernatant liquid was decanted and the precipitate suspended in sufficient 16.7 per cent NaCl solution (20 gm. NaCl per 100 ml. H<sub>2</sub>O) to make one liter volume. The pH was adjusted to 6.0 whereupon all the precipitate peptized to give a clear sol. The pH was again adjusted to 4.5 and the suspension centrifuged for 20 minutes. The precipitate was peptized by 16.7 per cent NaCl solution at pH 6.0 and made up to 500 ml. volume with the solvent. Sediment forming at this point was centrifuged out since the preliminary experiments had shown it to be relatively inactive. The pH was then adjusted to 4.5 and the suspension centrifuged for 20 minutes. The precipitate was peptized by the 16.7 per cent NaCl solution, made up to 250 ml. with solvent and the pH adjusted to 6.1. Again there was considerable sediment which was centrifuged out. Activity determinations were made on the final liquid and on the original rennet extract. The solution was stored in a cold room at 2° C. Portions were dialyzed "free" of NaCl as needed.

*Determination of rennin activity:* Rennin activity was determined by pipetting 10 ml. portions of fresh, raw skim milk into suitable test tubes in a constant temperature water bath held at 40°+ C., allowing three minutes to come to 40° C., adding 0.25 ml. CaCl<sub>2</sub> solution (containing 94.5 mgm. CaCl<sub>2</sub>) to each tube, letting stand three minutes more and then adding 0.5 ml. of the rennin sol of different dilutions until a coagulation time of approximately 10 minutes was obtained. The CaCl<sub>2</sub> treated milk had pH 5.75-5.80 by glass electrode pH meter. Actual coagulation times were determined by stop watch. The activity of each sol was calculated on the basis of the dry organic matter required to produce coagulation in 10 minutes. The organic matter in the various milk coagulating sols was de-

<sup>1</sup> Kindly furnished by the Chr. Hansen's Laboratory, Inc., Milwaukee, Wisconsin.

terminated on suitable volumes (*e.g.*, 30 ml.) after dialysis in viscose sausage casings against running distilled water, any residual NaCl being deducted after a volumetric Cl determination using a standard AgNO<sub>3</sub> solution and K<sub>2</sub>CrO<sub>4</sub> indicator.

**Determination of peptic activity:** Peptic determinations on the purified and original dialyzed samples were carried out as follows: To a 50 ml. centrifuge tube containing five ml. of two per cent egg albumin solution, which had been boiled for ten minutes and cooled, were added 15 ml. of 0.05 N HCl solution, and two ml. of the enzyme preparation. After incubation for three hours at 40° C. the mixture was centrifuged and tyrosine determined by the Folin-Marenzi (9) method on 20 ml. of the supernatant liquid. Blanks were run on the coagulated, acidified, incubated egg albumin sol containing boiled pepsin sol. Color comparisons were made with a Cenco Photometer, using a set of standards of crystalline tyrosine for comparison. Known concentrations of crystalline pepsin prepared from Armour's 1-10,000 pepsin<sup>2</sup> produced a tyrosine liberation curve from the egg albumin under standard conditions, from which the amount of pepsin per unit weight of dry rennin preparation could be estimated. The method has the advantage of detecting minute amounts of pepsin in an unknown sample, it being possible to determine accurately 10 µg. pepsin, the limit of sensitivity being about 5 µg. The rennin preparations had to be dialyzed because NaCl interferes with the color formation. Anson's (8) procedure for pepsin differs in that he used a phenol reagent for determining tyrosine, and employed a colorimeter for the color comparison.

#### PROPERTIES OF THE PURIFIED RENNIN

The results of the rennin and peptic activity determinations on the original extract and purified rennin are shown in table 1. It is seen that the rennin activity was increased 4.55 times and the peptic activity decreased to

TABLE 1  
*Enzyme activity of original rennet extract and purified rennin*

Enzyme Preparation	Rennin activity determinations				Peptic activity determinations			
	Wt. dry substance in 0.5 ml.	Total dilution	Clotting time	Calc. Activity*	Wt. dry substance in 2.0 ml.	Wt. tyrosine liberated	Estimated wt. pepsin	Estimated conc. pepsin**
	<i>mg.</i>	$\times 10^6$	<i>min.</i>	$\times 10^6$	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	%
Original rennet	6.98	14.3	9:00	15.9	1.984	1.654	0.073	3.68
Purified rennin	1.47	68.0	9:25	72.3	12.240	1.005	0.028	0.23

\* Million parts of milk clotted per part of rennin in 10 minutes.

\*\* Concentration of pepsin on dry weight basis.

<sup>2</sup> First crystallizations using the method of Northrop (10).

6.25 per cent of that of the original extract. The purified rennin was 99.77 per cent "pure" from the standpoint of pepsin contamination.

Further examination of the properties of the purified rennin showed that it behaved as a globulin, being (a) soluble in dilute salt solutions, (b) precipitating at pH 4.5, (c) insoluble in saturated salt solutions, and (d) precipitating out upon electrodialysis. This is in marked contrast to previous reports in the literature (1, 2, 3). The reddish brown color was not found to be associated with the rennin activity as believed by Richardson and Palmer (10). Most of this colored material was found to be left in the first supernatant liquor obtained at pH 4.5, where the major portion of the original peptic activity was separated. Another impurity found to be present was sodium benzoate, which crystallized out as benzoic acid at pH 2.5.

Cataphoresis experiments were carried out on the purified rennin using the apparatus described by Briggs (12). A small quantity of the dialyzed sol was diluted, 1 to 10 with distilled water, the pH adjusted to the desired value by adding 0.1 N NaOH solution and the undispersed particles followed cataphoretically with the microscope. Between pH 6.0 and 7.5, the zone studied, there was a progressive increase in zeta potential. At pH 6.5 rennin and calcium caseinate exhibited approximately the same magnitude of negative zeta potential, namely,  $-14.25$  mv. and  $-13$  to  $-16$  mv., respectively. These results seem to make untenable the conclusion (10) that the isoelectric point of rennin is pH 6.9 to 7.0 and that at pH 6.5 a positively charged rennin micelle exists. A more complete report of electrophoretic studies of rennin action on caseinate sols will be given in another paper.

Very little loss in activity was noted after three months' storage of the purified rennin in 16.7 per cent NaCl solution at  $2^{\circ}$  C.

#### DISCUSSION

Previous investigators (3) have objected to the use of commercial rennet extracts from which to prepare purified rennin. Our experiments indicate that such objections are not valid if the nature of the major impurities is recognized and suitable methods employed for their removal. In our experience dialysis and centrifuging will remove the major impurities which are not removed with the foreign proteins by suitable salting out procedures at controlled pH. A rennet extract such as we employed already represents a highly concentrated sol of milk coagulating enzymes in comparison with the crude extracts of calves' stomach mucosa used by other investigators, which are certain to have been much more highly contaminated with mucin and other foreign substances.

The relative milk coagulating activity of rennins purified by various investigators has no exact significance. Not only has the substrate been different but in none of the investigations were the precise optimum condi-

tions determined or employed either with respect to the type of milk substrate or the relations between pH and Ca ions, all of which are known to affect the rapidity of coagulation and also its completeness. The problem is no doubt further complicated by the presence of pepsin for which the optimum conditions no doubt differ. These criticisms apply to our own study in which the dry organic matter of the starting material apparently already had an activity about 3.5 times that of the purified rennin of Tauber and Kleiner (3), if one compares activity by the method we employed with activity by their method. Each study, therefore, is more or less a unit in itself with respect to comparative rennin activities and will show only the degree of concentration of rennin obtained in that study.

The method described in this paper of purifying rennin, at least from the standpoint of pepsin contamination, seems to have the following advantages over methods previously described:

- (a) Speed of purification is not an essential factor.
- (b) No inorganic solvents are employed which markedly denature the rennin.
- (c) The pH is controlled within the zone of greatest stability (4.5 to 6.5) so that pepsin does not inactivate the rennin as is the case in highly acid and highly alkaline solutions.
- (d) The use of electrolytes is an advantage, rather than detrimental (as when organic precipitants are employed), for they improve dispersability, and act as bactericidal agents.
- (e) The yield of coagulating activity is high, both actual and in relation to original dry organic matter. We recovered 30 per cent of the original activity in seven per cent of the original dry organic matter. It is not possible to say how much of the activity lost was due to pepsin removed.
- (f) The method does not necessitate drying and thus avoids loss in activity which may accompany the denaturation involved in this procedure.
- (g) The purified rennin retains its activity for many weeks at low temperature in approximately one-half saturated NaCl solution.

#### SUMMARY

A stable purified rennin sol was prepared from a commercial rennet extract by means of an isoelectric precipitation procedure. One part of dry rennin coagulated 72,300,000 parts of fresh raw skim milk at pH 5.75-5.80 (obtained by added  $\text{CaCl}_2$ ) in 10 minutes at 40° C., which was 4.55 times that of the dry organic matter of the original extract. The peptic activity, measured by a method which estimates quantitatively 10  $\mu\text{g}$ . crystalline pepsin, was only 6.25 per cent of the original dry organic mixture. The rennin was therefore 99.77 per cent "pure" from the standpoint of peptic activity. The purified rennin exhibited the properties of a globulin. Its isoelectric point in 16.7 per cent NaCl solution was pH 4.5. The rennin sol,

freed from NaCl by dialysis, showed a progressive increase in negative zeta potential from pH 6.0 to 7.5, and at pH 6.5 showed about the same negative zeta potential as calcium caseinate particles. Rennin and calcium caseinate, therefore, do not exhibit opposite electropotentials at the pH of milk.

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# THE PHOSPHATASE TEST—EXTENT OF USE IN NORTH AMERICA<sup>1, 2</sup>

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In order to determine how universally the phosphatase test is being used in North America, 454 questionnaires were mailed out to individuals in official city, state, provincial, county, private, and milk plant laboratories. (List furnished through the courtesy of the American Public Health Association Committee on Standard Methods for the Examination of Dairy Products.)

A total of 281 questionnaires or 62.1 per cent of the number mailed were returned. Fifty-five laboratories reported that they were not using the test. Eighteen of these were no longer active or were not doing milk work. Of the other 37 laboratories not using the phosphatase test, 17 were official city laboratories (16 U. S., 1 Canada), 5 were State Laboratories (one of these reported it did not use the test routinely, but did not state the modification which was occasionally used), 9 were private laboratories, and 6 were plant laboratories.

It is interesting to note the rapidity with which the phosphatase test has been taken up by various laboratories charged with the responsibility of safeguarding the quality of milk supplies.

Laboratories reporting as using the phosphatase test or one or more of its modifications are classified in table 1.

In a similar though less comprehensive survey made in 1940 (1) there

TABLE 1  
*Laboratories reporting the use of the phosphatase test*

Kind of laboratory	Number
State laboratories (including Hawaii and Puerto Rico)	32
County laboratories	14
City laboratories (including private laboratories doing contract city work)	122
Private laboratories	8
Milk plant laboratories	35
Canadian provincial laboratories	7
Canadian city laboratories	7
Canadian milk plant laboratories	1
Total	226

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<sup>1</sup> Presented at the Fifth Session of the 70th Annual Meeting of the American Public Health Association, October 16, 1941.

<sup>2</sup> A detailed tabulation of the data furnished by the various laboratories reporting the use of the phosphatase test is available in mimeographed form and may be had by writing to the author.

were 22 city laboratories which reported that they were using the phosphatase test, from which no report was received in the present survey. One laboratory returned the blank questionnaire with a notation of having filled out such a questionnaire once before, and if the information was desired again, it would be forthcoming.

In view of the fact that we have no record of a laboratory reporting as discontinuing the test once they have used it, we may assume that there are at least 22 additional laboratories in the United States which are using the test.

#### PASTEURIZATION TEMPERATURE

The official pasteurization temperature and time reported by the various laboratories as in effect in their city or state are tabulated in table 2. Reports were obtained from 133 cities and 41 states, including Hawaii and Puerto Rico.

TABLE 2  
*Official pasteurization time and temperature reported*  
Method of pasteurization

Low temperature long hold				High temperature short hold			
Temperature	Time	States	Cities	Temperature	Time	States	Cities
°F.	min.	No.	No.	°F.	sec.	No.	No.
140	30		3	160	15	16	42
140	40		1	160	16		1
140 -145	30	1	7	160	30		1
142	30	8	22	160	15-30		1
142 -144	30		1	160-163	15		1
142 -145	30	7	11	160-165	10-15		1
142 -146	30		1	161	15		1
142.5	30	1	2	161	16		4
142.5-145	30		1	161-162	15-16		1
143	30	18	52	162	12		1
143 -144	30		1	162	15		1
143 -145	30		6	162	17		1
143.5	30	1	14	165	15		2
144	30		3	165	16		1
145	30	1	8	165	30	1	

TABLE 3  
*Official pasteurization temperature and time reported for Canadian*  
provinces and cities

Temperature	Time	Provinces	Cities
°F.			
140-145	20-30 min.	1	....
143	30 min.	1	2
143-146	25-30 min.	1	..
145	30 min.	2	5
162	20 sec.	.....	1
163	20 sec.	1	.....

Four states did not report an official pasteurizing temperature and time, and only 17 of the 41 states reported an official temperature and time for high temperature, short hold pasteurization.

From Canada reports for official pasteurization temperature and time were received for 5 provinces and 7 cities. These are reported in table 3.

Although the legal pasteurization temperature may be below 143° F. in 49 cities and 17 states, a number of questionnaires contained the information that the prevailing temperature employed in the plants was 143° F. or higher, and the use of the phosphatase test had been instrumental in getting the plants to use these higher temperatures.

#### PHOSPHATASE TEST USED

The various phosphatase tests used by the 226 laboratories reporting the use of the test are classified in table 4.

TABLE 4  
*Classification of the phosphatase tests used*

Test used	Number of laboratories
Original Kay and Graham	10
Kay, Graham & Neave modification	5
Gilcreas & Davis modification	36
Scharer field test	82
Scharer laboratory test	33
Scharer field and laboratory test	35
Scharer field and Gilcreas & Davis	13
Scharer laboratory and Gilcreas & Davis	2
Scharer field and laboratory and Gilcreas & Davis	3
Scharer field and Kay, Graham & Neave	1
Scharer laboratory and Kay & Graham	2
Scharer field and Kay, Graham & Neave and Kay & Graham	1
Kay & Graham and Leahy	1
Kay, Graham & Neave and Gilcreas & Davis	1
Leahy	1
Total	226

The majority of the laboratories followed the directions as given in the 7th Edition of Standard Methods for Milk Analysis (2). There were 38 laboratories, however, that used some modifications of these directions.

Most of the modifications were in connection with the Scharer field test, and dealt generally with incubation time. Seven laboratories reported increasing incubation time to 20 minutes. Six used 30-minute incubation. Three used one hour incubation. One used 30-40 minutes, and two used 15 minutes.

The modifications of the various tests reported are summarized in table 5.

The use of a centrifuge in the place of filtration reported by two laboratories using the Gilcreas and Davis test should prove to be of considerable value in cutting the cost of making the tests and also in reducing the time

TABLE 5  
Summarization of the modifications of various tests reported

No. laboratories	Incubation		Other modifications
	Time	Temperature	
Modifications of the Scharer field test			
1	30-40 min.		Uses 2 drops B.Q.C.
1	10 min.	56° C.	Stands 15 min. after adding B.Q.C.
1	10 min.	56° C.	
6	30 min.		
6	20 min.		
3	1 hour		
1	15 min.	110° F.	0.2 cc. B.Q.C. used. No alcohol extraction
1	15 min.	100° F.	
2	10 min.	110° F.	
1	20 min.	105° F.	
Modifications of the Scharer laboratory test			
1			Uses a photometer
1	1.5 hrs.		Uses different color standards
1	12.0 hrs.		
1			
Modifications of the Scharer Field and Laboratory test			
1			Uses 0.1 cc. of (0.04 gm. B.Q.C. in 25 cc. alcohol)
1			Increase incubation time
1			Uses crystalline buffer instead of solution, also acidified alcoholic solution of B.Q.C.
Modifications of the Gilcreas & Davis test			
2	...	...	Use centrifuge instead of filtration
1			Uses ½ quantities and increase incubation
1			Uses photometer
Modifications of the Kay and Graham tests			
2	4.0 hrs.	...	
Modification of the Key, Graham and Neave test			
1	...	...	Report use of Na <sub>2</sub> CO <sub>3</sub>
Modification of Kay and Graham and Kay, Graham & Neave Test			
1			Uses photometer

involved when a large number of samples are run. It could also be applied to all of the various laboratory tests, such as the Kay and Graham, Kay, Graham and Neave, and the Scharer laboratory tests.

PRODUCTS REPORTED TESTED BY THE VARIOUS LABORATORIES USING  
THE PHOSPHATASE TEST

The majority of the laboratories use the test for milk or for milk and

cream only. Quite a few apply the test to ice cream and chocolate milk also. Only a few use it for butter and cheese. See table 6.

TABLE 6

*Summary of the use of the various tests and the number of samples tested by each method*

Product tested	Labora- tories total No. testing	Total samples tested by the various tests as follows:						
		Leahy	Gil- creas & Davis	Kay & Gra- ham	Kay, Gra- ham & Ncave	Two meth- ods other than Scharer	Two or more, one or more of Scharer	One or more of Scharer only
Milk	201	2,260	25,635	2,409	4,133	3,474	37,699	155,060
Cream	142	41	6,274	1,692	2,283	64	4,074	81,378
Milk and Cream*			1,485	16,851			7,701	73,886
Ice Cream**	35		22				499	6,007
Chocolate Milk	47		32				121	7,732
Butter	6							1,131
Cheese	5							423
Buttermilk	1			2				
Skim milk	1				12			
Cremo (half & half)								102
Dairy Products†	2		332					19,296
Total Samples		2,301	33,782	20,954	6,428	3,538	50,094	345,015

\* Not reported separately

\*\* Including frozen desserts

† Kind not designated

Number of samples run by the various groups of laboratories and the per cent positive reported are given in table 7. (Reports generally were for 1940.)

In addition, the following number of samples was reported tested, but the per cent positive was not stated.

Milk	36,700
Cream	900
Milk and Cream	20,995 (not reported separately)
Ice Cream	875
Chocolate Milk	250
Dairy Products	27,239 (kind not reported)
Total	86,959

Thus, a total of 462,115 samples of dairy products was reported tested by the various laboratories. Most of the reports were for the year 1940.

In conclusion, this survey has shown that the phosphatase test has been adopted with a surprising rapidity by many laboratories in North America. Nearly a half million samples were reported tested during the past year.

The Scharer Field Test was the one most generally used. In answer to question 6, "Have you found the Scharer Field Test reliable?", 123 replied "Yes," 10 "No," 3 said it was "Fair," 1 "Doubtful," and 1 "Limited."

TABLE 7

*Summary of samples tested by the various laboratories and the per cent of positive tests found*

Product tested	Kind of laboratory										Total	
	State <sup>1</sup>		County		City		Private		Milk plant		Canadian	
	Samples tested <sup>2</sup>	% pos.	Samples tested	% pos.	Samples tested <sup>3</sup>	% pos.	Samples tested	% pos.	Samples tested <sup>4</sup>	% pos.	Samples tested <sup>5</sup>	% pos.
Milk	26,719	4.29	5,603	13.67	82,984	2.97	2,060	2.71	74,181	0.62	6,960	6.51
Cream	4,950	3.17	979	3.88	18,364	4.97	1,435	1.60	61,172	0.57	1,111	4.32
Milk & Cream <sup>6</sup>	3,000	0.53	1,878	3.24	41,761	1.37	511	5.67			5,462	3.16
Milk, cream, Choc. milk <sup>7</sup>												
Ice Cream	650	7.84	2	0.00	2,512	20.72	260	0.38	12,930	0.49	4,300	7.69
Choc. milk	954	1.99	2	100.00	1,664	1.38	30	0.00	3,024	0.10	53	5.66
Butter	623	32.26			1,997	2.05	250	1.20	3,191	1.78	342	0.00
Cheese	15	0.00							258	35.27		
Cremo (half & half)									408	0.49		
Skim milk									12	0.00	102	1.96
Buttermilk											2	0.00
Dairy Products <sup>8</sup>					2,445	9.83						
Total	36,911	4.27	8,464	10.24	151,727	2.96	4,546	2.68	155,176	0.61	18,332	5.54
											2,445	9.83
											375,156	2.48

<sup>1</sup> Includes Hawaii and Puerto Rico

<sup>2</sup> An additional 11,750 tests were reported but the per cent positive was not stated

<sup>3</sup> An additional 27,239 tests were reported but the per cent positive was not stated

<sup>4</sup> An additional 45,995 tests were reported but the per cent positive was not stated

<sup>5</sup> An additional 1,975 samples were tested but the per cent positive was not stated

<sup>6</sup> Per cent positive not reported separately

<sup>7</sup> Kind not designated

In reply to the other part of question 6, "Does it compare in reliability with other tests when samples are incubated under controlled conditions for 20 or more minutes?", 47 stated "Yes," 3 "No," 1 said "30 minute incubation was," 2 said "Nearly," and 1 "Usually."

One state laboratory stated that they used the Scharer Field and Laboratory tests for detecting underpasteurization, and used the findings as a basis for prosecution.

The answers to question 10, "Have you noticed any decrease in the number of samples improperly pasteurized as compared to the first time tests were made?" indicates that the phosphatase test has been of decided value in insuring proper pasteurization. One hundred thirty-seven answered "Yes" while 32 answered "No." The majority of those stating the amount of decrease reported that it is more than 50 per cent.

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## METABOLISM STALLS

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In balance trial and digestion trial work at the Virginia Agricultural Experiment Station prior to 1938 the experimental animals were kept in a room with a level concrete floor where attendants caught the excreta on shovels and in buckets. To carry out a trial without loss of excreta on the floor was extremely difficult. Since our work is entirely with cows, we found it impossible to collect satisfactorily with rubber ducts. Furthermore a stove, which was located on one side of the room, overheated the cows nearest the stove, causing them to pant, and allowed the attendant of the cows on the side of the room away from the stove to become chilled. How much this affected the results of digestion trials could only be surmised.

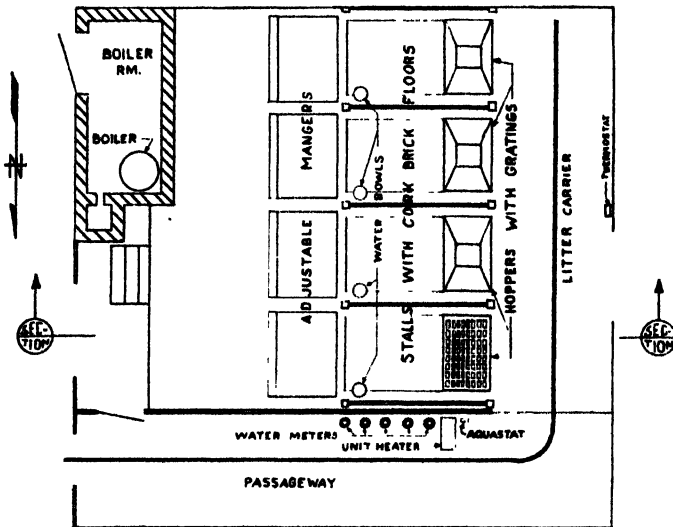
To avoid these conditions and to reduce the cost of continuous calcium and phosphorus balance trials the metabolism stalls described in this article were built. A basement room 22 feet square was used for this purpose. This room has three outside exposures; the east side adjoins the main stable. As is shown in the floor plan, the metabolism stalls are arranged so that cows can be led into this room from the west side. The second illustration shows the sections through the first stall.

Four stalls are elevated three feet above the floor level by means of retaining walls and dirt, excavated from the rear of the stalls, used for filling. Over this foundation a slab of reinforced concrete was poured with sunken areas in each stall for laying cork brick floors. In the rear of each stall, a storm grating was fitted with a copper funnel underneath to direct the excreta into thirty-gallon garbage cans. Because of corrosion by sulphates, these copper funnels had to be replaced by galvanized iron. Doors which can be raised to allow the attendant to remove the collection cans were hinged to the outside walls in a position level with the rear of the stall.

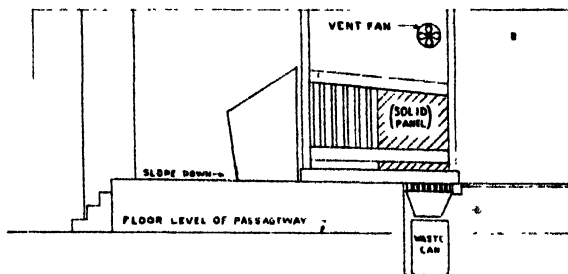
These stalls were separated by concrete curbs over which wooden stall partitions were located. Wooden frames covered with sheet metal were made to fit the rear of the stall and to direct the excreta downward to the storm grating. The rear half of the wooden stall partitions was also covered with sheet metal to prevent the excreta from one cow from reaching the adjoining stall. When these stalls were used for digestion trials where the feces and urine must be collected separately, the splash boards were removed from the rear of the stalls to allow the attendant to have access to the cow. A litter carrier track installed at the rear of these stalls connected with the track system of the barn. Wooden mangers were provided of such

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a width that they would telescope into the front of the stall between the partitions thereby adjusting the length of the stall to the individual cow. Short pieces of pipe were sunk into the concrete at intervals of three inches into which door bolts fastened to the manger could be inserted, preventing



PLAN



SECTION

DRAWN BY R. P. JOHNSTON

animals from shoving the mangers forward and backward. The details of manger construction are given in a previous paper (1).

Ventilation is provided by a "Jamesway" ventilator in the south wall of the room. This ventilator fan is regulated by thermostatic control, which starts the fan when the temperature reaches 56° F. and stops it when the temperature falls to 52° F.

The room is heated by a hot water supply boiler. A "Trane" unit heater, installed in the hot water line, is so located as to direct the heat past

the end of the partition which separates the metabolism stalls from the work room from which the metabolism stalls are entered at the rear. A wall thermostat controls the unit heater. The water in the system is circulated by a "Thrush" circulator located in the boiler room which is directly ahead of the metabolism stalls. The water circulator in turn is operated by a reverse acting aquastat in the returning hot water line between the unit heater and the circulator. An overhead expansion tank allows for the necessary change in volume due to heating of the water and a check valve prevents hot water from being forced back into the cold water line which feeds the system.

The combined action of the "Jamesway" ventilator and of the heating system results in a very uniform temperature and in low humidity except when the outside air is excessively humid.

Four "Trident" water meters measure the water which flows to water cups placed in the four stalls. Another meter measures the water which might be used for washing the stalls. This would be necessary if water from the pipe line were used for washing purposes as the water is very "hard." During the two years which the stalls have been in use, distilled water has been used for washing the stalls daily before the excreta is weighed. The use of the meters to the water cups makes it possible to estimate the amount of calcium supplied to each cow through the water.

A severe case of mastitis developed in one cow. Since occasional cases of mastitis develop in the main herd the metabolism stalls may not have been the cause in this case. However the storm gratings are good conductors of heat and help to chill the udder. A chimney effect is produced by the funnel and a draft results. This might be prevented by a canvas attached to the funnel and gathered tightly around the can by an elastic band.

#### SUMMARY

Four metabolism stalls were constructed with storm gratings placed in the rear of each stall and with funnels underneath which direct excreta into garbage cans. A fan ventilator with a thermostatic control regulates the humidity quite satisfactorily. A hot water boiler and unit heater provided with a water circulator controlled by a reverse acting aquastat provide satisfactory heat.

These stalls have been used for two years for balance trials, successfully eliminating the need for constant attendants.

When used for digestion trials in which the feces and urine are collected separately, these stalls helped to eliminate errors by making it possible to recover quantitatively either feces or urine which the attendant failed to catch.

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# THE CAROTENOID CONTENT OF MILK FAT FRACTIONS

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The separation of milk fat by fractional crystallization into different fractions was recently reported in connection with the study of the effect of the properties of the fat on lipolytic activity in milk (11). This study indicated a definite variation between the fat constants of different fractions. However, that paper did not contain data concerning the carotenoid content and the melting point of the fractions.

Since it was observed that the color intensity of the fractions varied inversely with the temperature of crystallization, it was thought desirable to learn if there is a relationship between the carotenoid content and the properties of the fractions.

For this study the carotenoid content of different fractions was determined by Hand and Sharp's method (8), and is reported in mg. per liter of melted fat. The melting points were determined using the A.O.A.C. (1) method.

The data presented in figure 1 indicate that there is an inverse relationship between the carotenoid content and the melting points of different fractions. They also show that the carotenoid values curve is definitely parallel to that showing the iodine number.

The iodine numbers show a definite tendency for unsaturated acids to concentrate in the liquid fractions.

The Reichert-Meissl numbers show that the volatile soluble acids are also concentrated in the fractions having lower melting points. However, this tendency to concentrate in the liquid phase practically disappeared below 15° C., while the tendency for unsaturated acids and carotenoid to concentrate in the liquid phase continued down to the lowest temperatures studied. These results suggest, therefore, that the solubility of carotenoid in the fraction is to a large extent dependent upon the concentration of unsaturated fats.

Several investigators have studied the seasonal variations in the carotene and vitamin A content of the milk fat (2, 3, 7, 10, 13). These studies indicated that with the change from dry feed to pasture, both the carotene and the vitamin A content of milk fat increased rapidly. However, it is generally recognized that the feed of the cows exerts a definite influence on the chemical properties of milk fat. Hunziker, Mills, and Spitzer (9) were the early workers to show that the change from dry feed to pasture caused an abrupt increase in the iodine numbers and a decrease in Reichert-Meissl numbers of milk fat.

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Although these experiments are not directly comparable, nevertheless it appears that the seasonal variations in carotene and vitamin A content seem to parallel those in iodine numbers of milk fat.

In view of the fact that only a small fraction of ingested carotene appears in the milk fat (3, 12), the above observations, and the data presented in figure 1, suggest the possibility that the absorption of carotene by an animal body and consequently the carotene and the vitamin A content in

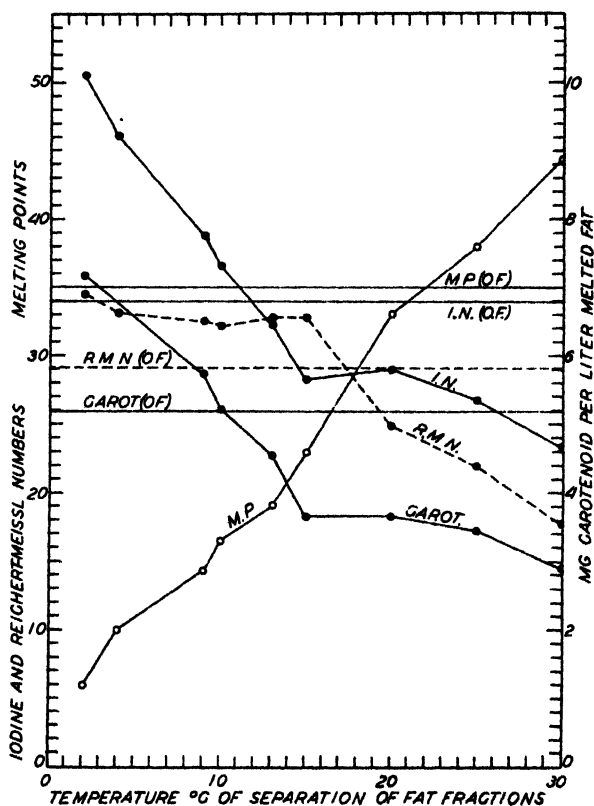


FIG. 1. Fat constants and carotenoid content of different milk fat fractions. (M.P.—melting points; I.N.—iodine numbers; R.M.N.—Reichert-Meissl numbers; O.F.—original fat.)

the milk fat could be affected not only by the carotene content of the feed but by the character and amount (5, 6, 14) of the fat in the diet as well.

Some interesting results were obtained with respect to the keeping qualities of milk fat fractions. These fractions, after two years' storage in an incubator at 4° to 5° C., were tested and scored. It was found that generally the intensity of the oxidized flavor varied inversely with the iodine number and the carotenoid content of the fractions. The fraction having the lowest melting point and which also showed the highest iodine number and carotenoid content appeared to be the best one with respect to flavor.

These observations seemed to indicate that the carotene might be the substance responsible for the reduction in the susceptibility of the fat fraction to oxidized flavor. However, in view of the recent work of Brown *et al.* (4) there is a possibility that some other substance associated with carotene is concentrated in the liquid fraction and is responsible for this effect.

The extreme low temperature fractions might well serve as the starting point in an attempt to identify the highly unsaturated acids and the anti-oxidant of butter fat.

#### SUMMARY

A study was made of the relationship between the carotenoid content and the physico-chemical properties of different milk fat fractions.

The data indicate an inverse relationship between the carotenoid content and the melting points of the fractions. They also indicate a definite relationship between the carotenoid content and the iodine numbers of the fractions.

The data suggest that the efficiency of absorption of carotene by an animal from its feed might be influenced by the degree of unsaturation of the fat present in the feed.

The flavor score of different fractions at the end of two years' storage at 4°-5° C. revealed that the intensity of the oxidized flavor varied inversely with the carotenoid content of the fractions. It appears that the substances responsible for the reduction in the susceptibility of the fat to oxidized flavor are concentrated in the liquid fraction.

The extreme low temperature fractions might well serve as the starting point in an attempt to identify the highly unsaturated acids and the anti-oxidant of butter fat.

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# THE EFFECT OF HIGH-TEMPERATURE SHORT-TIME FOREWARMING OF MILK UPON THE HEAT STABILITY OF ITS EVAPORATED PRODUCT

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The stabilizing effect of high temperatures of forewarming on milk which is to be concentrated and sterilized has been pointed out by Grindrod (2). During the development of his impact process of sterilization he observed that, when high velocity steam was injected into a stream of milk, stabilization to the heat of sterilization occurred. Made by his process evaporated milk had a heat stability four times that obtained by the usual treatment. Grindrod attributed the stabilizing action of the jets of steam largely to the apparent redispersing action upon coagulated albumin, calcium salts, and to a lesser extent coagulated casein. He states, "It appears to be very desirable, if not absolutely essential, that the steam should heat the material instantly by direct contact as distinguished from processes in which part of the milk is heated by direct contact with a hot body and the rest becomes heated by convection or distribution due to its being mixed with the previously heated material" (3). Grindrod noticed that these evaporated milks of high stability often showed a body too thin for a commercially acceptable milk.

Recently, methods of rapidly heating fluids to high temperatures in tubular heaters without allowing the fluid to come in direct contact with steam have been perfected (5). Since equipment of this type was made available in these laboratories it was believed that a detailed study of the effect of high heat treatment or "high temperature forewarming" of milk upon the heat stability of evaporated milk made from it would be of value to the industry. The results reported in this paper are concerned with the effect of high temperature forewarming with a 25-second holding period upon the heat stability of evaporated milks of 18 per cent solids-not-fat content.

## EXPERIMENTAL

The milk used in this investigation was produced by the Bureau of Dairy Industry herd at the National Agricultural Research Center, Beltsville, Maryland. The cows were healthy except for a few cases of chronic mastitis. No milk was received from cows suffering from acute mastitis.

Skim milk was used in the early work. After it was noted that skim milk did not always produce the same type of heat stability curve as whole milk, most of the experiments were conducted with whole milk.

The fresh whole milk was standardized by the Babcock test and a hydrom-

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eter reading (4) to a fat: solids-not-fat ratio of 1:2.29. It was then forewarmed under the desired experimental conditions, concentrated under 28 to 29 inches of vacuum to less than one half its initial weight, quickly heated to 80° C.,<sup>1</sup> homogenized at once at 2500 pounds per square inch pressure, cooled and standardized with water to 26 per cent total solids. The skim milks were forewarmed, concentrated, cooled and standardized with water to 18 per cent solids content. Approximately 70 pounds of whole milk or skim milk was used for each sample.

The control samples were prepared in the same way except that the milk was forewarmed to 95° C. and maintained at this temperature 10 minutes. This forewarming treatment corresponds to the usual commercial procedure. A few samples of milk were also forewarmed at 65° C. for 10 minutes. All samples referred to in this paper as having been forewarmed below 100° C. were so treated with thorough agitation in a steam jacketed kettle.

Samples reported as forewarmed above 100° C. were high-temperature forewarmed by forcing the milk through 0.18-inch I. D. stainless steel tubing with a reciprocating pump. The capacity of this pump was 1.5 gallons per minute but the rate of flow of the milk through the tubing was governed by the relationship between the resistance to this flow and the pressure required for the milk to by-pass through an homogenizing valve. The actual rate of flow of milk through the tubing was approximately 1 gallon per minute.

The heating coil was installed in an insulated metal chamber into which high pressure steam could be admitted through a reducing valve. The holding period depended on the seconds required for the liquid to pass through a known length of tubing wound around a jacketed metal post. The cooling coil was immersed in rapidly flowing tap water.

Temperature readings were obtained by means of thermocouples screwed into tees in the stainless steel tubing, a cold junction, a millivoltmeter and suitable connections. Four thermocouples were employed. One was inserted between the pump and the heater, another between the heater and the holding coil, the third at the end of the holding coil and the fourth at the end of the cooling coil.

After the thermocouples had been standardized it was only necessary, when a certain temperature was desired, to divide that number by a conversion factor to determine the corresponding reading on the scale of the millivoltmeter.

For the most part the milk was heated to the desired temperature in 4 seconds, held 25 seconds and cooled to a temperature of less than 38° C. in 4 seconds.

Heat stability determinations were made by heating the samples of concentrated milk in small cans (208 × 208) in a pilot sterilizer until coagulation was observed. The reel of the sterilizer was equipped with 2 chutes

<sup>1</sup> °F. = (°C. × 1.8) + 32.

which held 10 cans each and which were built so that one or more cans could be removed at any time without disturbing the remaining samples. The stability data were recorded in minutes of heating required to produce the first signs of coagulation. Data were accurate to approximately  $\pm 4$  per cent of the coagulation time. The sterilization temperature for whole milk was  $115^{\circ}\text{C}$ . One hundred twenty degrees centigrade was used for skim milk in order to avoid long heating periods.

When stabilizing salts were used, they were added as standard solutions to 130 ml. of milk measured into each can. A total of 1 ml. of salt solution or water was added per can to keep the milk solids concentration constant. Milks receiving 1 ml. of  $\frac{1}{3}\text{ M Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  or 1 ml. of  $\frac{1}{2}\text{ M CaCl}_2$  per 130 ml. of milk received an equivalent of about 14 ounces and  $6\frac{1}{2}$  ounces of the dry salt respectively per 1000 pounds of milk.

### RESULTS

The data presented in table 1 show the magnitude of the stabilizing effect produced by high forewarming temperatures. The heat stability of the test samples was generally about 2 and occasionally as much as 6 times greater than that of the control samples.

Data representing the effect of some variations in forewarming fresh whole milk upon the heat stability of its evaporated product have been plotted in figure 1. Observations on the body and physical condition of

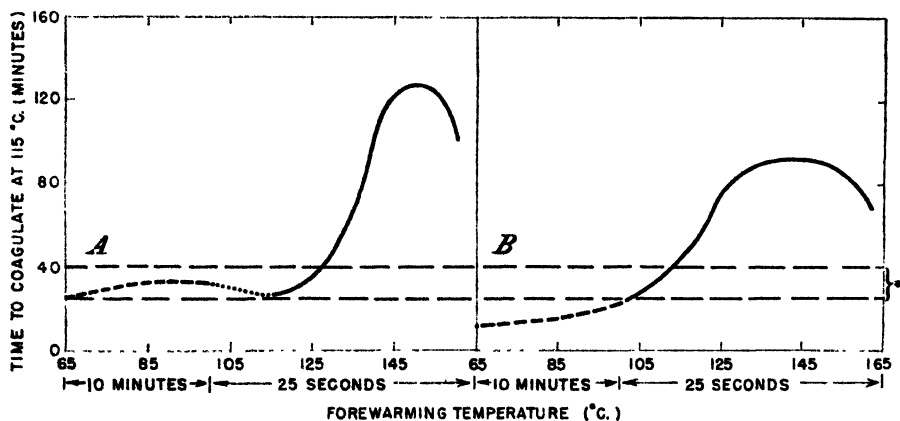


FIG. 1. The effect of variations in forewarming upon the heat stability of two types of evaporated whole milk. Broken lines indicate low temperature, long time forewarming. Solid lines indicate high temperature, short time methods. (\* Stability range required for production of milk of good body.)

many samples after sterilization indicated that milks falling within the stability range of 25 to 40 minutes possessed a commercially acceptable body. Milks below this range showed a slight grain or were of excessive viscosity. Milks with a stability greater than 40 minutes were thin at the end of an 18 minute sterilization period.

The destabilizing effect of the fat phase and the effect of dispersal of the fat by homogenization of the concentrated milk were studied. Some data have been plotted in figure 2. Skim milk was always more stable than whole milk. Generally, the whole milk and skim milk curves were of the same type

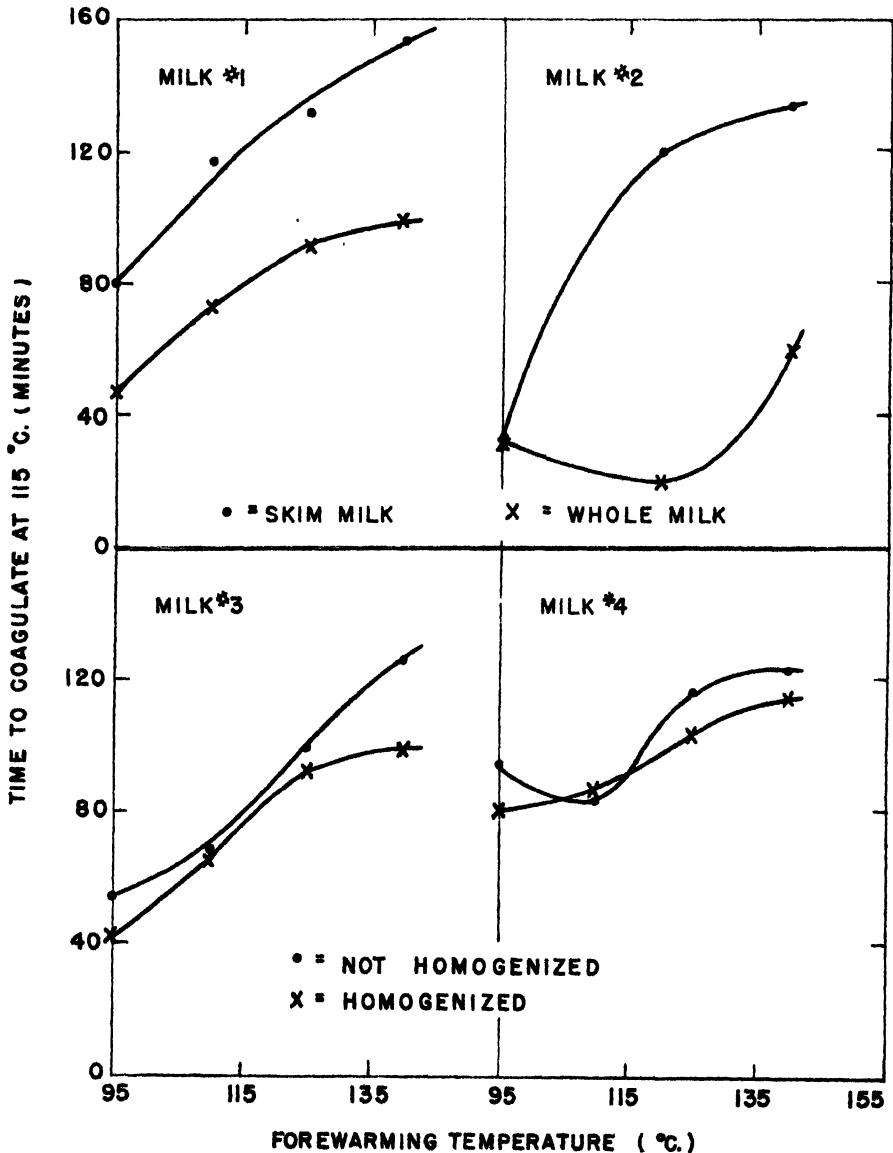
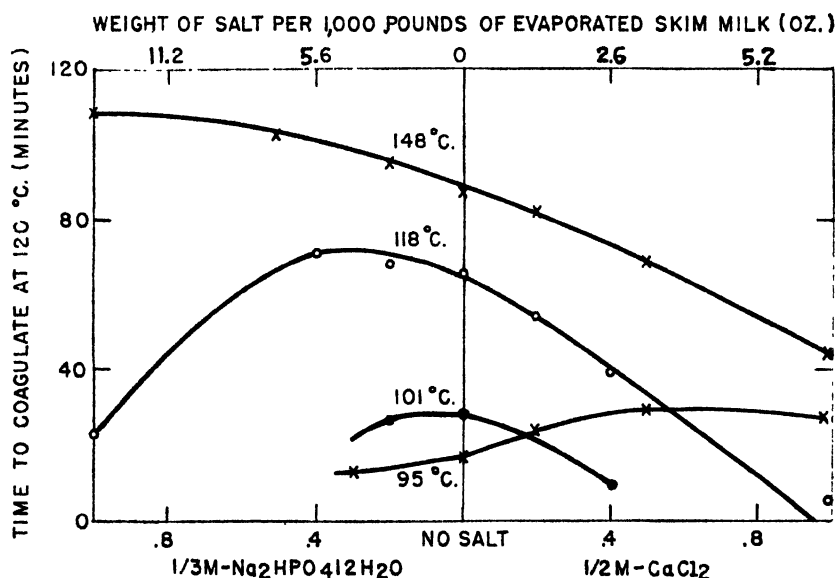


FIG. 2. Some effects of milk fat and its homogenization upon the heat stability of four evaporated milks made from milk forewarmed to different temperatures. Portions of milks #1 and #2 were separated before forewarming to obtain the skim milk. Milks #3 and #4 were each divided into 2 parts after forewarming and concentrating but before homogenization.

but milk #2, figure 2, shows a whole milk destabilized by forewarming to 120° C., while its skim milk was greatly stabilized by the same forewarming treatment.

It is known that homogenization decreases the heat stability of evaporated milk (1). Data plotted in figure 2, milks #3 and #4, indicate that, while homogenization generally destabilizes the milk, this effect may under some conditions be negligible. The greater stability of homogenized milk #4 over the unhomogenized sample at 110° C. forewarming is so small as to be within the limits of experimental error.

Although each batch of concentrated whole milk made during the course of this work was heated to 80° C. before homogenization, this pre-homogeni-



STABILIZING SALT ADDED PER 130 ML. OF EVAPORATED SKIM MILK (ML.)

FIG. 3. The effect of stabilizing salts and forewarming treatments upon the heat stability of evaporated skim milks.

zation heat treatment actually had but slight stabilizing action. A large batch of whole milk forewarmed to 130° C. was condensed and divided into four parts, which were heated to different temperatures before homogenization at 2500 pounds per square inch pressure. Samples homogenized at 37° C., 50° C., 65° C. and 80° C. coagulated when sterilized at 115° C. in 35 minutes, 35 minutes, 37 minutes and 38 minutes respectively.

The stabilizing effect of added salts and of high forewarming temperatures is shown in the graphs of figures 3 and 4. Figure 3 indicates that high forewarming shifts the curve of a normally forewarmed skim milk, which is stabilized by calcium to curves which show some stabilization by phosphate.

It has been noted (6, 7, 9) that a calcium-stabilized milk could be changed to one stabilized by phosphate through the addition or the development of acid in the milk. This condition may be again noted by comparing the fresh and aged samples of skim milk #2, figure 4.

Milks of excessive acidity coagulated when subjected to high temperature forewarming, but normal milks of good quality withstood temperatures of 150° C. to 160° C. without coagulating.

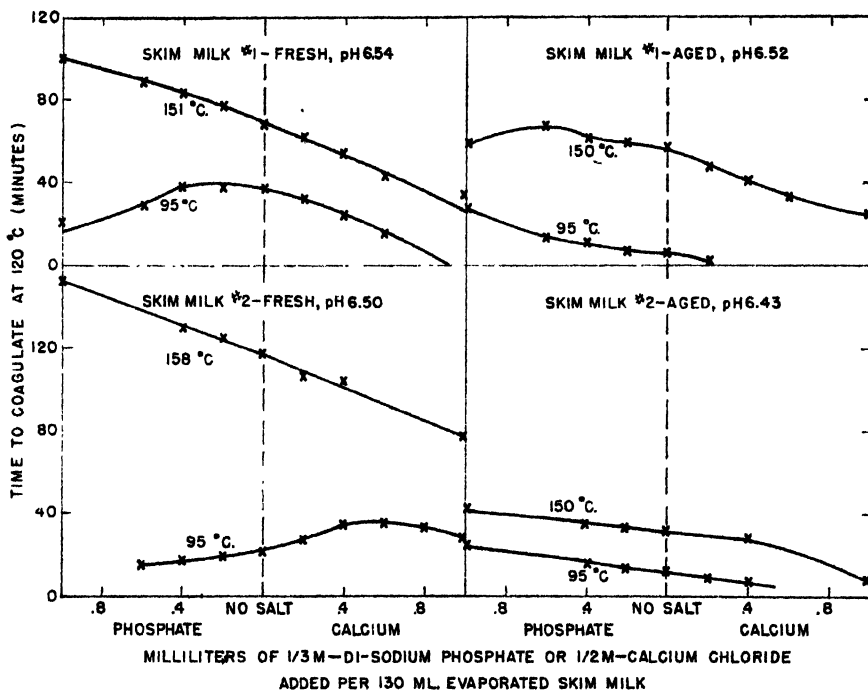


FIG. 4. The effect of stabilizing salts upon the heat stability of two evaporated skim milks. A portion of each milk was processed when fresh and another portion after a ripening period. The pH values were determined before processing.

The color, flavor and pH of milks forewarmed to 140° C. for 25 seconds and to 95° C. for 10 minutes did not show important differences but there were undesirable changes in color and flavor and an increase in acid intensity when temperatures above 140° C. were used.

#### DISCUSSION

Table 1 includes representative data obtained from milks produced at intervals during one year. No milks were found which did not show greater stability than the control when they were forewarmed to a temperature of 140° C. or higher for 25 seconds. The degree of stabilization brought about by high-temperature forewarming was not the same in various samples of

TABLE 1

*The effect of forewarming treatment upon the heat stability of evaporated milk. Control samples were held at 95° C. for 10 minutes. All other samples were held 25 seconds at the indicated temperatures*

Skim milk. T.S. = 18%			Whole milk. T.S. = 26%		
Date	Forewarming temperature	Time to coagulate at 120° C.	Date	Forewarming temperature	Time to coagulate at 115° C.
	°C.	min.		°C.	min.
12/23/40	95	8*	3/17/41	95	33
	150	26*		120	30
12/31/40	95	10		140	105
	150	76	4/ 8/41	157	121
1/ 6/41	95	28		95	33
	152	67		120	21
1/13/41	95	27	4/14/41	140	60
	145	88		95	43
1/16/41	95	15		120	35
	101	24	4/29/41	140	65
	118	60		95	36
	133	67		110	55
	141	72		125	92
	148	75		140	98
1/31/41	95	22	5/ 7/41	95	81
	158	117		110	86
				125	104
2/14/41	95	12		140	114
	150	32	5/26/41	95	20
2/18/41	95	37		130	85
	112	58		140	92
	151	68		150	91
4/29/41	95	80†	6/ 2/41	95	81
	110	117†		140	130
	125	132†	6/23/41	95	37
	140	154†		140	84
			8/11/41	95	35
				150	70
			9/25/41	95	44
				120	98
				140	94
			10/ 1/41	95	30
				130	54
			10/27/41	95	28
				120	16
				140	58
			11/ 3/41	95	36
				140	45
			12/11/41	95	74
				140	93

\* Time to coagulate at 125° C.

† Time to coagulate at 115° C.



milk. The average values for the stability of the whole milks forewarmed to 95° C. and to 140° C. were 45 and 86 minutes respectively.

The range of forewarming temperature which can be used to produce a milk of proper body depends upon the nature of the forewarming-stability curve of the milk. Figure 1 shows two types of forewarming curves. Milk A fell within the proper stability range when it was forewarmed at any temperature between 65° C. and 127° C., while milk B showed the required stability over the range, 102° C. to 113° C. From available data it is reasonable to assume that the forewarming-stability curve for every milk is different. As a milk is aged and acid is developed the entire curve is lowered. Factors such as changes in salt equilibrium shift the curve to the right or left. If curve A is shifted downward its steepest part can be made to pass through the 25 to 40 minute stability area over a range of only 2° C. in forewarming temperature. An operator, confronted with such a milk, would find it difficult to estimate what forewarming temperature should be used to obtain the required stability. The manufacturer's problem is further complicated by his desire to secure the same type of body in every batch. This would probably make it necessary for him to adjust the permissible stability in a somewhat narrower range than the 25 to 40 minute period given in figure 1.

It is probable that greater difficulty will be encountered in obtaining proper body in a high-temperature short-hold forewarmed milk than in a normally forewarmed milk because the optimum high forewarming temperature must be more closely estimated. This can be done by setting each day's high forewarming temperature after consideration of the previous day's run. When no clue is available on the stability of a milk, the safest procedure would be to forewarm to 130° C.-145° C. for 25 seconds.

Experimental work of the type reported in this paper is more easily conducted with skim milk than with whole milk. After consideration of the data presented in figure 2, however, it seemed desirable to use whole milk until such time as the relationship between the fat phase, heat stability and various manufacturing procedures was better understood. The experiments in which skim milk was used were conducted early in the investigation. Recent trials indicate that the general relationships shown in figures 3 and 4 apply also to whole milk.

The stabilizing effect which may be obtained by selection of the optimum high forewarming temperature was found to be greater than the stabilizing influence secured by addition to the milk of the most favorable quantity of calcium or phosphate salt.

The following tabulation of data from figures 3 and 4 should be of interest. It shows the average heat stability of the control and high-temperature forewarmed samples together with the average heat stability of these

milks when the amount of stabilizing salts giving optimum stability was used.

Forewarming treatment	Time to coagulate when no stabiliz- ing salts were used	Time to coagulate when the opti- mum quantity of stabilizing salts was used
	<i>minutes</i>	<i>minutes</i>
Control—95° C.—10 min.	19	32
Heated to approximately 150° C.—25 sec.	74	93

The milk used in this work was equivalent to market milk and was of a different grade than that received in some condenseries. The effect of a slight development of acidity in the Beltsville cows' milk upon the nature of the flash forewarming results was investigated. The skim milk of table 1, dated 1/31/41 (also figure 4, No. 2) had a reaction of pH 6.50. After part of it was held at 1° C. two weeks and then at room temperature several hours, its reaction was pH 6.43. When this milk was processed on 2/14/41, its heat stability was again greatly increased by high-temperature short-hold forewarming. The sample of whole milk in table 1, dated 5/26/41, was aged 2 hours at 32° C. with added lactic starter and subsequently held at 1° C. overnight before subjecting it to the usual manufacturing procedure. The ripening treatment changed its reaction from pH 6.58 to pH 6.56. Stabilizing salts would have been necessary to produce a commercial evaporated milk of satisfactory body from the control sample but the high-temperature forewarmed samples showed more than ample heat stability. The effect of high-temperature forewarming upon the heat stability of a skim milk in which a slight amount of acid has developed is also shown in figure 4, No. 1.

The results on mildly aged milks indicate that forewarming to the proper high temperature is an effective means of stabilizing concentrated milk toward sterilization. Unless the milk has developed an excessive amount of acid which it may be necessary to neutralize, stabilization by high-temperature forewarming would appear to be better practice than stabilization by salts. In some cases it is possible that both stabilizing methods might be necessary.

Stabilization by heat during forewarming possesses this disadvantage over salt stabilization of the concentrated milk—the operator must guess from past experience what the optimum forewarming treatment of the raw milk should be. No certain and accurate tests are available for determining this. Stabilization by salts is carried out by adding small increments to numerous samples of the concentrated milk just prior to sterilization. Pilot sterilizer runs are made and the exact amount of salt needed is accurately determined.

Available studies (8) on the effect upon heat stability of mixing different grades of milk indicate that if 2 milks of the phosphate-stabilized type are mixed, the milk of higher stability will increase the stability of the poorer milk somewhat in proportion to the quantity of more stable milk used. It is probable that a milk condensery could adjust the stability of its milk by sterilizing the proper blend of normally forewarmed and high-temperature forewarmed, concentrated milk. The heat stability and body of many evaporated milks could be controlled in this way without the use of stabilizing salts. High-temperature forewarming 25 per cent to 50 per cent of the milk received would, in most instances, be sufficient to make a stable concentrated mixture suitable for sterilization.

During the course of a year's experimental work, there was consistent improvement in the heat stability of high-temperature forewarmed milks over milks forewarmed below 100° C. It is not to be expected that the data could be exactly duplicated when milks are used which are produced under different seasonal or geographical conditions. It is believed, however, that the basic relationships observed in this work will be found to exist wherever high-temperature forewarming methods are applied.

A study of the development and maintenance during storage of a smooth body, viscous enough to retard fat separation in evaporated milk, was not made during the course of this work. The effect of different forewarming treatments of whole milk upon the viscosity of its evaporated product during storage is being investigated.

The remarkable heat stability of high-temperature forewarmed milks of 26 per cent total solids content indicates the possibility of processing milks of higher solids concentration. Accumulated data show that the time a milk is held at the high-forewarming temperature exerts a great influence upon its subsequent heat stability. A general study of the relationships between conditions of forewarming, solids content and heat stability is in progress and will be reported later.

#### SUMMARY

1. The heat stability of evaporated whole milk of 26 per cent total solids content was increased as much as 6 times that of control samples by high-temperature short-hold forewarming the fresh milk. The control samples were forewarmed to 95° C. (203° F.) and held 10 minutes; the test samples were forewarmed over a range of temperatures from 101° C. (213.8° F.) to 165° C. (329.0° F.) with a heating time of 4 seconds, a holding time of 25 seconds and a cooling time of 4 seconds.

2. The relationship between the high forewarming temperature and the heat stability of evaporated milk differs with each milk. A study of this relationship indicates that the high-forewarming temperature required to produce an evaporated milk of a certain desired viscosity may be within

limits of 2° C. (3.6° F.) for one milk or within limits as wide as 60° C. (108° F.) for another milk. Milks forewarmed to produce excessively high stability will be too thin, while those with too low stability will be rough after sterilization.

3. Use of the optimum high-forewarming temperature brought about, in the milks tested, a greater increase in heat stability in the evaporated milk than could be attained by the addition of the optimum quantity of stabilizing salt to a normally forewarmed milk.

4. High forewarming should be a useful commercial procedure for increasing the heat stability of milks which are difficult to sterilize without the addition of stabilizing salts. When a coming-up time of 4 seconds and a holding time of 25 seconds are used, the optimum high-forewarming temperature for most milk will probably fall between 120° C. (248° F.) and 140° C. (284° F.).

#### ACKNOWLEDGMENT

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# THE BACTERIOLOGY OF BRICK CHEESE. II. COMPARISON OF WASHED-CURD AND CONVENTIONAL METHODS OF MANUFACTURE<sup>1</sup>

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## INTRODUCTION

The bacteriology of Brick cheese during manufacture by the conventional procedure has been discussed in a previous report (2). In these experiments cheese made by this method always developed excessive acidity when the moisture content remained above 39 per cent, even when different starters and cooking temperatures were used. While some experienced cheese makers have been able to make a "sweet" cheese containing this much moisture, many cheese makers have had difficulty in doing so. The fermentation of the lactose in the cheese by lactic acid bacteria during manufacture and storage usually ceases only with the exhaustion of the milk sugar. In our experiments, when the moisture content of the cheese remained above 39 per cent, fermentation of all the lactose lowered the pH to about 4.8, and an acid cheese with a short, crumbly body resulted. In an effort to prevent the development of excessive acidity, a washing procedure was introduced to remove part of the lactose from the curd before draining. Comparisons of the changes in pH and bacterial numbers in the cheese during manufacture by both the conventional and washed-curd procedures will be considered here.

## METHODS

Methods of sampling curd and cheese and the procedure of bacteriological analysis have been described previously (2). Incubation temperatures of 22° C. and 47° C. were used for the cultural counts. Garey (1) established that *Streptococcus lactis* and *Streptococcus thermophilus* could be separated quantitatively in this way, since at 22° C. the growth of *Str. thermophilus* was inhibited but that of *Str. lactis* was not; while at 47° C. the reverse occurred.

The milk used was from the mixed milk supply of the University of Wisconsin Creamery. The conventional procedure for making Brick cheese was that described by Wilson and Price (6) and by Langhus (3). The washed-curd method differed from the conventional procedure only in the treatment of the curd between cutting and dipping. The following washing procedures were tried:

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“One-time wash”—40 to 45 pounds of whey were removed per 100 pounds of milk and replaced with an equal volume of water at the temperature of setting.

“Mild wash”—25 to 28 pounds of water per 100 pounds of milk were added to the curd plus whey in the vat. Then 50 to 55 pounds of whey were removed and replaced by an equal volume of water warmed to 90° F.

“Double wash”—40 to 45 pounds of whey were removed per 100 pounds of milk and replaced by 80 to 85 pounds of water per 100 pounds of milk.

The double wash procedure was used for the cheese described here because it was known to control the high-acid defect; but cheese made by this method frequently developed defects due to insufficient acidity. The mild wash method was introduced to leave more sugar in the curd, so that the acidity would increase slightly.

For the determination of the rate of disappearance of lactose from the cheese, slivers about one-eighth inch thick were sliced longitudinally from the center of a plug from the side of the loaf, placed on three by five inch index cards, and then heated at 104° C. for three to four hours. The degree of brownness in the heated slivers due to caramelization of the lactose indicated the amount of sugar present at any given sampling period. In recording the appearance of the cheese slivers, the relative degrees of browning were indicated by different numbers of plus signs, as shown in the footnote to table 1.

## RESULTS

### *Development of the Washed-Curd Method*

The washed-curd method was developed to regulate the sugar content of the curd and thus control the final acidity of the cheese. A pH of 5.0 to 5.15 was usually the safest range to insure a cheese that was not too acid yet would not show defects due to too low acidity.

Analyses of cheese made by the conventional method showed that the lactose content of the curd at dipping was usually about 6.5 per cent of the dry matter. Fermentation of this amount of sugar lowered the pH to about 4.8, which was much too acid to permit normal ripening. The “one time wash” and the “double wash” procedures removed approximately half the lactose and permitted maintenance of the moisture content of the cheese at a much higher level than the legal limit without excessive acidity, but the curd developed an unnatural “corky” feeling before dipping. The “mild wash” procedure removed slightly over one-third of the lactose with no harm to the curd and the quality of the cheese. This procedure allowed an excess of 2 to 4 per cent moisture over the legal limit without danger of a sour flavor and a weak or crumbly body.

*Bacterial Numbers, Acidity, Moisture Content and Lactose Content of Cheese Made by the Conventional and Washed-Curd Methods*

*Cheese made with Streptococcus lactis.* Representative data on two lots of cheese made from the same batch of raw milk by the conventional and double washed-curd processes with *Streptococcus lactis* starter are shown in table 1. In the cheese made by the conventional method there was a steady

TABLE 1

*Comparison of the effects of the conventional and washed-curd methods of manufacture on the bacteriological and chemical changes in cheese made from raw milk with one per cent Streptococcus lactis starter and cooked to 106° F.*

Age of cheese		Acidity pH	Numbers of bacteria per gram (000 omitted). Cultural counts at 2 different incubation temperatures		Moisture content (per cent)	Residual lactose*
Days	Hrs.		22° C.	47° C.		
Conventional method						
0	2	5.77	110,000	1.5		..
0	4	5.25				..
0	6	5.12				..
0	8	4.96				..
0	10	4.88	6,050,000	2.5		+
1	..	4.80	10,500,000	8	46.10	+
7	..	4.87	3,700,000	45	41.43	-
14	..	4.90	3,000,000	10		-
21	..	4.76	400,000	2.5	40.40	-
28	...	4.81	1,500,000	3	41.60	-
35		4.78	3,300,000		40.80	-
42		4.78	700,000	1.5	40.60	-
Wash-curd method						
0	2	5.88	1,050,000	12		..
0	4	5.27	.....	...		..
0	6	5.15	.....	...		..
0	8	5.12				..
0	10	5.12	3,750,000	85		+
1	...	5.10	4,000,000	90	45.64	-
7	....	5.22	3,550,000	50	41.22	-
14	..	5.24	250,000	40	40.90	-
21	..	5.16	150,000	20	41.10	-
28	...	5.27	350,000	15	40.70	-
35	.....	5.22	240,000		40.10	-
42	....	5.27	600,000	17	40.00	-

\* Designations of relative degrees of browning:

		Approximate percentage of lactose in the dry matter
Dark brown	+++	Above 2
Medium brown	++	1-2
Brown	++	0.3-1
Light brown to mere trace	+	Less than 0.3
White	-	None

and rapid drop in pH to 4.80 at one day, and little change thereafter because of complete disappearance of the lactose after the first day. The bacterial



counts showed a rapid increase in numbers of the starter organisms parallel to the decrease in pH up to one day, little change for two weeks, and a gradual diminution thereafter. The numbers of organisms capable of growing at 47° C. were rather low throughout the ripening period. These consisted of *Str. thermophilus*, *Str. bovis*, *Str. fecalis* and *Str. liquefaciens*, present originally in the milk. This lot of cheese was criticized for an acid, bitter flavor, short body and excessively close texture.

With the exception of those in pH the course of changes in the washed-curd cheese was not significantly different from that described for the conventional method. As might be expected, the lactose disappeared more quickly from the washed-curd cheese whose pH dropped more slowly than it did from the cheese made by the conventional method. The minimum pH of 5.1 was reached at one day, after which it could go no lower because of the lack of sugar. This cheese lacked the undesirable high acidity of that made by the conventional procedure, but was criticized for lack of flavor.

TABLE 2

Comparison of the effects of the conventional and washed-curd methods of manufacture on the bacteriological and chemical changes in cheese made from raw milk with one per cent *Streptococcus thermophilus* starter and cooked to 106° F.

Age of cheese		Acidity pH	Numbers of bacteria per gram (000 omitted). Cultural counts at 2 different incubation temperatures		Moisture content (per cent)	Residual lactose*
Days	Hrs.		22° C.	47° C.		
Conventional method						
0	2	5.45	9,500	140,000		
0	4	5.32				
0	6	5.34				
0	8	5.21				
0	10	5.20	9,500	280,000		++++
1		5.22	13,000	135,000	44.64	++++
7		5.11	475,000	10,000	40.80	++++
14		5.03	700,000	2,000	39.40	+++
21		5.01	800,000	3,000	38.70	++
28		5.04	1,200,000	24,000	39.20	++
35		4.99	500,000	3,000	39.20	++
42		4.98	680,000	900	39.00	++
Wash-curd method						
0	2	5.75	7,000	14		
0	4	5.62				
0	6	5.56				
0	8	5.53				
0	10	5.52	11,000	90,000		++
1		5.50	185,000	59,500	45.67	++
7		5.30	1,500,000	30,000	42.00	+
14		5.42	900,000	49,000	43.10	-
21		5.45	1,200,000	42,000	40.10	-
28		5.47	1,200,000	50,000	39.50	-
35		5.43	1,400,000	90,000	40.60	-
42		5.42	800,000	40,000	39.80	-

\* See footnote to table 1.

Both lots of cheese were lower in moisture than is desirable. However, it is apparent that the pH in the cheese made by the conventional procedure would have been much lower had the moisture content been near the legal limit.

*Cheese made with Streptococcus thermophilus.* Representative data on two lots of cheese made from the same raw milk by the conventional and washed-curd processes with *Streptococcus thermophilus* starter are shown in table 2. In the cheese made by the conventional method there was a very rapid increase in the starter organisms during the first few hours after manufacture, then a sharp decrease. Low temperature organisms (those growing at 22° C.) increased slowly but persisted in large numbers throughout ripening. The pH eventually went as low as 4.98, and considerable lactose remained. This cheese was criticized for unclean and bitter flavors, short body and an open texture.

In the cheese made by the washed-curd method the changes in numbers of bacteria capable of growing at both 22° C. and 47° C. followed the same general trend during the period of increase as was found in the conventional cheese. However, the numbers of both types persisted at higher figures for a longer time in the washed-curd cheese. The lactose content of the washed-curd cheese naturally was lower and the sugar disappeared entirely after one week; hence the pH remained at 5.3-5.4 throughout ripening. This condition allowed the development of anaerobic spore-forming bacteria in great abundance, and blowing of the cheese resulted. The final product was criticized for a very undesirable bitter flavor, tough, rubbery body, and an extremely open texture.

*Cheese made with a combination of Streptococcus lactis and Streptococcus thermophilus.* The changes in the cheese made with *Str. lactis* and *Str. thermophilus*, as shown in table 3, followed the course usually taken in cheese made with a combination of the two starter organisms (2). In both the cheese made by the conventional method and that made by the washed-curd method the high temperature organisms were mainly the thermoduric starter bacteria. These decreased to insignificant numbers after one week, while the low temperature forms persisted longer and in much greater numbers. The pH of the cheese made by the conventional method dropped steadily until the lactose had almost disappeared at seven days, and very slowly thereafter. A very slight amount of lactose persisted near the rind of this lot of cheese throughout ripening, apparently due to inhibition of the lactic acid bacteria by the higher salt concentration in that area. The pH of the washed-curd cheese dropped steadily for one week, after which little further change occurred. The cheese made by the conventional method was criticized for excessive acidity and a short body; the washed-curd cheese had a satisfactory body but little flavor and a few pinholes.

A comparison of the three lots of cheese described above shows that the

TABLE 3

*Comparison of the effects of the conventional and washed-curd methods of manufacture on the bacteriological and chemical changes in cheese made from raw milk with a combination of 0.5 per cent Streptococcus lactis and 0.5 per cent Streptococcus thermophilus and cooked to 106° F.*

Age of cheese		Acidity pH	Numbers of bacteria per gram (000 omitted). Cultural counts at 2 different incubation temperatures		Moisture content (per cent)	Residual lactose*
Days	Hrs.		22° C.	47° C.		
Conventional method						
0	2	5.58	280,000	15,000		
0	4	5.29				
0	6	5.25				
0	8	5.26				
0	10	5.10	850,000	700,000		++
1	..	5.07	1,300,000	700,000	44.32	++
7	....	4.92	1,550,000	5,000	41.02	+
14	....	4.80	800,000	1,850	39.50	+
21	....	4.76	1,100,000	1,500	39.20	+
28	....	4.78	1,100,000	1,400	39.00	+
35	....	4.72	800,000	1,700	38.80	+
42	....	4.73	750,000	700	39.20	+
Wash-curd method						
0	2	5.58	220,000	31,500		
0	4	5.54				
0	6	5.43				
0	8	5.33				
0	10	5.27	1,450,000	350,000		++++
1	..	5.17	2,050,000	65,000	44.12	+
7	....	5.14	2,150,000	20,000	40.75	-
14	....	5.12	900,000	900	39.40	-
21	....	5.11	1,900,000	1,000	39.20	-
28	....	5.12	900,000	500	38.80	-
35	....			500		-
42	....	5.12	300,000	160		-

\* See footnote to table 1.

washed-curd method is successful in controlling the acidity, but may enhance other defects. The best results were obtained in both methods with a mixture of the starter organisms.

#### DISCUSSION

The process of washing Brick cheese curd with water before dipping originated in an effort to manufacture a product with a uniformly desirable flavor, body and texture containing as much moisture as legally allowable but lacking the usual acid defects of cheese with high moisture content. Preliminary studies of the starter organisms (1) had shown that this could not be accomplished by alterations of the manufacturing methods then being used. Fermentation of the residual lactose in high-moisture cheese made by the conventional procedure lowered the pH out of the range compatible with normal ripening, and a very acid cheese with a short, crumbly body and

close texture resulted. When *Str. lactis* was used as part or all of the starter the lactose disappeared completely within the first few days of ripening, and the acidity was high enough to prevent the development of undesirable bacteria. When *Str. thermophilus* was used alone as the starter it ceased growing a few hours after dipping, leaving considerable lactose in the cheese and the pH at a relatively high figure (5.30–5.45). The acidity did not increase until low temperature organisms (*Str. lactis* and others capable of growing at the curing temperature) developed and fermented the remaining lactose. These changes often required several weeks, thus giving ample opportunity to other organisms present in the milk to grow and produce undesirable changes in flavor and texture.

It was realized that a method must be developed to lower the pH rapidly to a point at which undesirable fermentations could not occur, but not too low to prevent normal ripening changes. This was done by removing part of the lactose by the curd washing process. Preliminary trials showed that washing more than once would not be practicable in the ordinary cheese plant because of the extra water and labor required; hence the wash water was applied only once. In the first experiments most of the whey (40–45 pounds whey per 100 pounds of milk) was removed first, and then replaced with an equal or greater quantity of water warmed to 90° F. Cooking was then carried on as usual. This procedure left about one-half the lactose, which was sufficient to yield the desired acidity, but had the disadvantage of causing the curd particles to mat when the whey was first drawn off. The curd prepared in this way always had an unnatural “corky” feeling when pressed together in the hand rather than the usual firmness characteristic of curd particles in the conventional method of manufacture.

The best product was prepared by the “mild wash” method. In this procedure about 25 pounds of water were added per 100 pounds of milk; then twice this amount of whey was removed and replaced with an equal volume of water. Thus sufficient liquid remained in the vat during washing to suspend the curd particles and prevent their matting. There was no undesirable effect on the curd particles. About 35 per cent of the lactose was removed by washing and the remainder disappeared as quickly as it did in the conventional method to give the desired pH (usually less than one day). Washing had no harmful effect on the activities of the starter bacteria. After six weeks of ripening the flavor of this cheese was clean and mild, the body was smooth and soft, and the texture was medium close. The smooth, soft body was attributable to the relatively high moisture content and to a greater degree of proteolysis than occurred in high-acid cheese.

It is recognized that most of these experiments were performed under laboratory conditions with milk of high quality; hence the methods cannot be recommended without reservation for all field conditions. It was generally observed that when a defect due to undesirable bacteria appeared in

cheese made by the conventional method, this defect was even more pronounced in cheese made from the same batch of milk by the washed-curd method. Differences in acidity of the cheese probably explain this observation. Many defects such as late gas formation by anaerobic spore-forming bacteria usually can be prevented by lowering the pH to 5.0. It is natural, then, if this or similar defects occur in conveniently made cheese with its relatively higher acidity that they would occur to an even more marked degree in cheese made by the washed-curd method with its lower acid content.

Usually, then, the washed-curd method satisfactorily controls the acid defect in Brick cheese, and with milk of good quality yields a desirable product. But with milk of poor quality the method cannot be recommended without modification. Any defect other than excessive acidity in cheese made by the conventional method is merely enhanced when the same milk is used with the washed-curd procedure. The success attained with the washed-curd method to date is sufficiently encouraging to warrant its trial under practical factory conditions, and it is believed that cheese makers will find it advantageous in producing a uniformly better product with a higher percentage yield.

#### SUMMARY

1. In an effort to remedy the acid defect common in high moisture Brick cheese a process was introduced for washing the curd with water before dipping. After this treatment enough fermentable lactose remained in the curd to lower the pH to about 5.00–5.15 in cheese with a moisture content of 40–42 per cent.

2. Of the several washing procedures tried the "mild wash" (25 pounds of water added per 100 pounds of milk, 50 pounds of whey removed and replaced with 50 pounds of water) gave the best product. The cheese made by this method had a mild and clean flavor, soft and smooth body and a medium close texture.

3. The washing process had no noticeable effect upon the rate of development of the starter bacteria as detectable by the cultural counts, but there was a slightly slower rate of acid formation in the washed-curd cheese. Acid formation ceased soon in the washed-curd cheese because of the exhaustion of the lactose. A combination of *Str. lactis* and *Str. thermophilus* was a better starter than either alone.

4. The occurrence of undesirable fermentations was more pronounced in the washed-curd than in the conventional cheese, due probably to the relatively lower acidity in the former. However, with milk of good quality undesirable fermentations did not appear when the mixed starter was used, and the washed-curd cheese was superior to the other in flavor and body as well as in moisture content.

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# THE BACTERIOLOGY OF BRICK CHEESE. III. THE BACTERIA INVOLVED IN RIPENING<sup>1</sup>

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## INTRODUCTION

The function of microorganisms in the manufacture of cheese of all types is well recognized. It has been pointed out (7) that certain lactic acid forming bacteria are used in the manufacture of Brick cheese because of the desirable effect of their acid production upon the nature of the curd and upon subsequent changes during ripening. Also, it is generally accepted that microorganisms are responsible for many of the physical and chemical changes that occur during the ripening of all types of cured cheese. This study was made to ascertain the nature of the bacterial flora in the interior of Brick cheese during ripening. The surface flora, which is believed by Langhus (14) to function chiefly in flavor production, is not considered here.

The literature on the ripening of hard cheese has been reviewed recently by Orla-Jensen (16). Studies have been made on the bacterial flora of cheese similar to Brick, but no reports have been found on the role of bacteria in curing Brick cheese. Studies on Tilsiter cheese (9, 10, 11, 19) showed the lactic acid bacteria predominant throughout the ripening period. During the earlier part the lactic acid streptococci were most numerous, but during the later part the numbers of rod forms multiplied considerably while the numbers of coccus forms diminished. Micrococci usually were present throughout ripening.

Dalla Torre (5) reported that changes in the bacterial flora of Bel Paese cheese were similar to those in Tilsiter. The lactic acid bacteria, particularly *Streptococcus lactis*, predominated throughout ripening, but rod forms increased as the cheese aged. A few yeasts and appreciable numbers of gelatin liquefying cocci also were present.

A close resemblance between the course of chemical and bacteriological changes in Wilster Marschkäse and in related types of cheese (Tilsiter, Bel Paese, and Brick) was shown by the investigations of Boysen (3). Lactic acid streptococci, usually *Str. lactis*, predominated in this cheese during all stages of ripening but rod forms appeared after two to three weeks and increased slowly thereafter. Micrococci were never found after one day by the ordinary plating methods.

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## METHODS

The methods of manufacture of Brick cheese have been described previously (6, 7). Analyses were made of cheese manufactured by the conventional and double washed-curd methods from raw and pasteurized milk with *Str. lactis* and *Str. thermophilus* alone and in combination as the starter cultures.

The organisms predominant in the cheese were isolated from the tube cultures prepared for colony counts (as described in 7) and from each temperature of incubation (22°, 37° and 47° C.). To remove the agar one arm of a sterile, small-bore, V-shaped glass tube was inserted to the bottom of the culture tube alongside the agar; then gentle blowing into the other arm forced the agar into a sterile Petri dish, where selected colonies could be picked into litmus milk. Incubation of the isolated cultures was at the incubation temperature of the tube from which the culture had been made. At least one colony of each kind of bacterium present was picked, and the number of colonies selected per kind of organism was in proportion to the total number of its colonies in the tube.

Litmus milk cultures showing growth were examined microscopically; then triplicate subcultures were made in fresh litmus milk, and these were incubated at 22°, 37° and 47° C., respectively. From the microscopic appearance, the action in litmus milk and the temperature of growth it was possible to place all but a few of the cultures in one of eight groups. From these groups 66 representatives were selected for identification, but only 52 are represented in tables 1 and 2. As nearly as possible the cultures of each group represented different lots of cheese. The following observations were made on part or all the cultures:

*Minimum and maximum temperatures for growth* were determined by incubating cultures growing in buffered glucose carrot-liver extract broth at 10°, 20°, 30°, 37°, 40°, 47°, 50° and 55° C., respectively, and observing microscopically at frequent intervals for the amount of growth. Since the optimum temperature of the lactobacilli is a specific differential characteristic, representatives of each rod culture growing in the above medium were incubated at the three available temperatures apparently nearest their optima. Turbidity measurements were made at frequent intervals with an Evelyn photoelectric colorimeter and the temperature which favored the most rapid rate of growth was considered the optimum.

*Methylene blue and salt tolerance* were determined by the methods of Yawger and Sherman (20).

*Ammonia formation from peptone* was tested by the method suggested by Ayers, Rupp and Mudge (2).

*Active proteolysis* was determined by examination of milk cultures for caseolysis.

**Hemolysis.** Plates of blood agar were inoculated by the spot method and examined for the type of hemolysis.

**Final pH in glucose broth.** The medium suggested by Ayers, Johnson and Davis (1) was used and the acidity measured with a Beckman pH meter.

**Final pH and titratable acidity in milk.** Each organism was inoculated into sterile skim milk and incubated at its optimum temperature. After 11 days the final pH was determined with a Beckman pH meter and the titratable acidity measured by titration with N/10 NaOH.

**Lactate utilization.** Each culture was inoculated into tubes of a broth medium containing 20 grams peptone, 5 grams yeast extract and 12 grams sodium lactate per liter and incubated at its optimum temperature. Unless a culture showed definite turbidity within one week it was not considered capable of utilizing lactate.

## RESULTS

The eight groups of cultures isolated as described above included five species of streptococcus and three of lactobacillus. Sherman's review on streptococci (17) and Orla-Jensen's monograph on lactic acid bacteria (15) provided most of the tests necessary for identification of the cultures, but the scheme given in Bergey's "Manual of Determinative Bacteriology," 5th Edition, was followed in the final classification and naming of each organism. Emphasis was placed on such observations as growth in lactate broth, action in litmus milk, growth in high concentrations of salt, production of acid in milk and growth at different temperatures, since all of these reflect something of the behavior of the organisms in the cheese. Sufficient additional tests were included to permit final identification in all cases except one or two, when two species were separable only by means of a single fermentation test.

**Lactic streptococci.** *Str. lactis* and *Str. cremoris* are listed by Sherman (17) as the lactic streptococci. These two species were not separable by the means used in this study; but since their differences are largely quantitative their separation was not considered of great importance. The six cultures identified as *Str. lactis* were selected from a group of 545 of this type, and were isolated from five lots of cheese. Cultures A-107, B-162, and E-111 were taken from cheese made with *Str. lactis* starter; the other three cultures were taken from cheese made with *Str. thermophilus* alone. Because the characteristics of *Str. lactis* are well known it was thought advisable to identify only a few of these cultures and place more emphasis on those which have been studied less extensively. All of the 545 cultures considered to be *Str. lactis* exhibited the usual diplococcus form in milk, showed the characteristic reduction of litmus milk before curdling, and grew only at temperatures considered typical for this organism.

Table 1 shows that the characteristics of the cultures identified as *Str. lactis* coincide rather closely with those described by Sherman. During the

TABLE 1  
Characteristics of the coccus forms

No. of culture	Growth at			Growth in presence of		NH <sub>3</sub> formed from peptone	Strong reduction	Lactate utilization	Final acidity in			Name of organism
	10° C.	40° C.	45° C.	6.5 per cent NaCl	0.1 per cent methylene blue				Glucose broth		Milk	
214	+	-	-	-	+	+	+	-	pH 4.00	pH 4.22	T.A.* 0.85	<i>S. lactis</i>
249	+	-	-	-	+	+	+	-	4.25			" "
A107	+	+	-	-	+	+	+	-	4.33	4.12	0.96	" "
B162	+	+	-	-	+	+	+	-	4.39	4.31	0.78	" "
C173	+	+	-	-	-	+	+	-		4.23	0.85	" "
E111	+	+	-	-	-	+	+	-	4.48	4.12	0.85	" "
B80	-	+	+	-	-	-	-	-	4.31	3.90	1.09	<i>S. thermophilus</i>
C110	-	+	+	-	-	-	-	-	4.64	3.98	1.03	" "
E38	-	+	+	-	-	-	-	-	5.01	4.00	1.00	" "
E84	-	+	+	-	-	-	-	-	4.28	3.96	1.09	" "
F88	-	+	+	-	-	-	-	-	4.80	3.94	1.08	" "
E146	-	+	+	-	-	-	-	-	4.60	3.94	1.10	" "
A120	-	+	+	-	-	+	-	-	4.77	4.75	0.59	<i>S. bovis</i>
B105	+	+	+	-	-	-	-	-	4.26	4.73	0.56	" "
C86	-	+	+	-	-	-	-	-	4.28	4.86	0.54	" "
D58	-	+	+	-	-	+	-	-	4.30	4.98	0.51	" "
D86	+	+	+	-	-	-	-	-	4.29	4.72	0.59	" "
180	+	+	+	+	+	+	+	+	3.89	4.11	0.96	<i>S. faecalis</i> †
240	+	+	+	+	+	+	+	+	4.03	4.48	0.71	" "
A70	+	+	+	+	+	+	+	+	4.27	4.64	0.66	" "
B147	+	+	+	+	+	+	+	+	4.15	4.92	0.53	" "
C50	+	+	+	+	+	+	+	+	4.23	4.64	0.66	" "
C151	+	+	+	+	+	+	+	+	4.28	4.67	0.64	" "
C184	+	+	+	+	+	+	+	+	4.22	4.64	0.66	" "
D185	+	+	+	+	+	+	+	+	4.28	4.59	0.70	" "
E151	+	+	+	+	+	+	+	+	4.20	4.57	0.67	" "
A177	+	+	+	+	+	+	+	+	4.22	4.73	0.92	<i>S. liquefaciens</i> ‡
C88	+	+	+	+	+	+	+	+	4.10	4.80	0.87	" "
D84	+	+	+	+	+	+	+	+	4.20	4.68	0.83	" "
E152	+	+	+	+	+	+	+	+	4.15	5.04	0.75	" "

\* Titratable acidity expressed as per cent lactic acid.

† No cultures proteolytic.

‡ All cultures strongly proteolytic.

early part of ripening this species appeared at times in numbers as high as two or three billions per gram of cheese, and even at the end of ripening often occurred in numbers of several hundred millions. The presence of so many *Str. lactis* organisms during ripening might lead to the belief that they function in the actual curing processes. Although very slight proteolytic action has been demonstrated for this species, most investigators consider acid formation to be its chief function in cheese manufacture and ripening.

*Viridans streptococci.* *Str. thermophilus* and *Str. bovis* were the viridans streptococci found in Brick cheese and identified as shown in table 1. The six cultures of *Str. thermophilus* represent a group of 119 isolations

from six lots of cheese; the five cultures of *Str. bovis* were selected from a group of 87 found in four lots of cheese. All cultures considered to be the same species agreed very well on the different tests, with the exception of a few minor variations between strains of *Str. bovis*. The main differential features between these two species were the changes in litmus milk and the final acidity produced in milk. In litmus milk cultures *Str. thermophilus* rapidly produced acid and coagulation, then slowly reduced the litmus either partially or completely. *Str. bovis*, on the other hand, grew much more slowly, seldom curdled milk at 37° C. but produced coagulation in four days at 47° C., and had very slight reducing power. *Str. thermophilus* produced much more acid in milk than did *Str. bovis*.

*Str. thermophilus* seldom was found unless it was added in the starter, and usually was present in numbers no greater than a few thousands per gram of cheese after two weeks, even when there had been two or three billions per gram present at 12 hours. *Str. bovis*, on the other hand, developed frequently from the original flora of the milk, especially in cheese made from pasteurized milk. It usually was found after the first week of ripening in numbers of one to fifty millions, and these numbers remained fairly constant throughout the curing period. The prevalence of this organism in cheese made from pasteurized milk is an indication of its high heat resistance, and helps to explain the high numbers of thermoduric organisms sometimes present in this type of product.

*Enterococci*. The members of the enterococcus group found in Brick cheese, chiefly in raw milk cheese, were *Str. fecalis* and *Str. liquefaciens*. Table 1 shows results of differential tests on 9 cultures representing a group of 98 isolations of *Str. fecalis* from 7 lots of cheese and on four cultures taken from a group of 19 isolations of *Str. liquefaciens* from 4 lots. The chief difference between the species was proteolysis, *Str. liquefaciens* showing active caseolysis in milk and liquefaction of gelatin, while *Str. fecalis* showed neither. Both organisms utilized lactate readily, a characteristic observed consistently only with these and one of the lactobacillus species.

The function of enterococci in the normal ripening of Brick cheese is not readily explainable. The marked proteolytic action of *Str. liquefaciens* indicates that it may take part in the ripening activities, but its irregular appearance and the unpleasant nature of its products make its action appear undesirable. When this organism grew occasionally to numbers of several millions per gram, a bitter flavor developed in the cheese. Although *Str. fecalis* sometimes was present in Brick cheese in numbers of several hundred thousands to a hundred million per gram, its function in the cheese was not determined. Its proteolytic ability is negligible and usually it was not isolated until all the lactose had disappeared. Hence it probably is not important either in protein breakdown or in acid formation. The ability of *Str. fecalis* to utilize lactate readily probably explains its appearance in consider-

able numbers in the cheese; and its occurrence in the numbers mentioned above certainly must have an effect on the other flora present and on flavor production.

*Lactobacilli*. Rod-shaped, lactic acid forming bacteria usually were detectable after one or two weeks in Brick cheese made from raw milk, but were not found in significant numbers in cheese from pasteurized milk. The species found were *Lactobacillus casei*, *Lactobacillus brevis* and *Lactobacillus lactis*, the first two appearing most consistently and the third in only a few instances. Table 2 shows some of the characteristics of the organisms that

TABLE 2  
Characteristics of the rod forms

No. of culture	Temperatures of growth in degrees C.			CO <sub>2</sub> formed in milk	Growth in presence of 0.1 per cent methylene blue	Milk curdled in 11 days	Strong reduction	NH <sub>3</sub> formed from peptone	Lactate utilization	Final acidity in			Name of organism
	Minimum	Optimum range	Maximum							Glucose broth	Milk	T.A.*	
186	10	28-35	45	-	-	+	+	+	-	4.00	3.94	1.13	<i>L. casei</i>
252	10	28-32	45	-	-	+	+	-	-	3.96	3.80	1.28	" "
A131	10	28-37	45	-	-	+	+	+	-	4.09	3.75	1.36	" "
A167	10	28-35	45	-	-	+	+	-	-	4.25	3.76	1.29	" "
B89	10	28-35	45	-	-	+	+	-	-	4.40	3.80	1.24	" "
B153	10	28-35	45	-	-	+	+	-	+	4.21	3.81	1.29	" "
C56	10	28-35	45	-	-	+	+	-	-	4.14	3.95	1.12	" "
C140	10	28-35	45	-	-	+	+	-	-	4.09	3.93	1.14	" "
D123	10	28-35	45	-	-	+	+	-	+	4.10	3.90	1.21	" "
D147	10	28-35	45	-	-	+	+	-	-	3.80	3.90	1.22	" "
D209	10	28-35	45	-	-	+	+	+	-	4.10	3.84	1.20	" "
216	10	28-32	40	+	-	-	-	+	+	4.90	5.14	0.47	<i>L. brevis</i>
237	10	28-32	40	+	-	-	-	-	+	4.19	4.81	0.60	" "
253	10	28-32	40	+	-	-	-	-	+	4.03	4.68	0.67	" "
B109	10	28-32	45	+	-	-	-	+	+	4.49	5.15	0.42	" "
C71	10	28-32	45	+	-	-	-	+	+	4.10	4.71	0.61	" "
D162	10	28-32	45	+	-	-	-	+	+	4.10	4.76	0.57	" "
E166	10	28-32	40	+	-	-	-	+	+	4.55	5.25	0.40	" "
B125	20	37-40	50	-	-	+	+	-	-	3.89	3.63	1.54	<i>L. lactis</i>
B162	20	37-40	50	-	-	+	+	-	-	4.10	3.60	1.58	" "
B200	20	37-40	50	-	-	+	+	+	-	4.00	3.61	1.56	" "
C108	20	37-40	50	-	-	+	+	-	-	3.90	3.71	1.51	" "

\* Titratable acidity expressed as per cent lactic acid.

belong to these species. The 11 cultures classified as *L. casei* represent a group of 90 isolations of this type. They had an optimum temperature of about 30° C. and curdled milk in two to four days with reduction of litmus. All cultures produced 1.1 to 1.5 per cent lactic acid in milk. The seven cultures of *L. brevis* selected from 44 isolations also grew best at 30° C., but they formed gas and only small amounts of acid in milk, which was not curdled even at the optimum temperature. Carbon dioxide was produced

in fairly large quantities and lactate was utilized by all cultures of this species. *L. lactis* was found in four lots of cheese, but never in very large numbers. Of the fourteen isolations of this organism, the four identified had high optimum (37° to 40° C.) and maximum (50° C.) temperatures, and produced a large amount of acid (1.5 to 1.6 per cent) in milk. At 37° C. these cultures caused rapid coagulation of milk and reduction of litmus in one to three days. The main differential features between the three lactobacillus species were the optimum and maximum temperatures of growth, carbon dioxide formation in milk, coagulation of litmus milk with reduction of the litmus, and the total acidity produced in milk. Considerable variation was noticed between the different strains of certain species in such tests as lactate utilization and the production of ammonia from peptone. None of them was able to grow in sterile skim milk containing 0.1 per cent methylene blue.

There are many references in the literature to the role of lactobacilli in cheese ripening (3, 8, 9, 10, 12, 13, 14, 16). Certain of these organisms have been shown to be able to produce significant proteolytic changes in cheese when they are present in large numbers. Hence, the detection of *L. casei* in numbers as high as 100,000,000 per gram indicates that this organism is important in the proper curing of Brick cheese. It is doubtful whether the other species of lactobacilli found in this study have much importance in the ripening, because their numbers were much smaller than were those of *L. casei* and their appearance more inconsistent. In fact, the gas production of *L. brevis* probably is undesirable in the cheese.

Table 3 summarizes the results of all the tests. It is believed that these tests, when accompanied by the usual observations of the microscopic appearance of the organisms and their action in litmus milk, yield sufficient information to serve as a scheme for the differentiation and identification of the bacteria that occur in Brick cheese.

*Anaerobic spore forming bacteria.* Throughout this investigation, at all seasons of the year, it was observed that agar culture tubes incubated at 22° and 37° C. frequently showed gas formation, at times sufficient to break the agar into small pieces. This condition prevailed only when the cheese had a relatively low acidity (above pH 5.20) and was shown to be due to anaerobic spore formers. These organisms grew readily in cheese made with *Str. thermophilus* alone because of its lower acidity. Because of the effect on the acidity, the use of pasteurized milk and the washed-curd manufacturing method also favored anaerobic gas forming bacteria. Cheese made under these conditions frequently showed cracks and splitting due to excessive gas formation, and a very undesirable fermented flavor after two to four weeks. These organisms have been reported previously in milk (7) and in cheese (3, 4, 7, 11). Since they are very resistant to heat it would appear that the best means of controlling them is to prevent, as far as possible, their entrance into the milk and to insure proper salting and acid production in the cheese.

TABLE 3  
Differential cultural characteristics of the organisms that comprise the bacterial flora of Brick cheese

Name of organism	Temperature ranges of growth—degrees C.			Milk curdled in 11 days	Growth in presence of 0.1 per cent methylene blue	Active proteolysis	Strong reduction	Lactate utilization	CO <sub>2</sub> formed in milk	NH <sub>3</sub> formed from peptone	Final acidity in		
	Minimum	Optimum	Maximum								Glucose broth	Milk	
<i>S. lactis</i>	8-10	30-35	37-40	+	+	-	+	-	-	+	pH 4.5-4.0	pH 4.3-4.1	T.A.* 0.75-0.95
<i>S. thermophilus</i>	15-20	37-40	47-52	+	-	-	-	-	-	-	4.8-4.2	4.0-3.9	1.00-1.10
<i>S. boum</i>	10-15	37-45	50-52	+	-	-	-	-	-	±	4.5-4.2	5.0-4.7	0.50-0.60
<i>S. fecalis</i>	8-10	30-40	45-50	+	+	-	+	+	-	+	4.3-3.8	5.0-4.5	0.50-0.80
<i>S. liquefaciens</i>	8-10	30-40	45-50	+	+	+	+	+	-	+	4.2-4.1	5.0-4.5	0.75-0.95
<i>L. casei</i>	8-10	28-35	40-45	+	-	-	+	±	+	±	4.4-3.9	4.0-3.6	1.10-1.50
<i>L. brevis</i>	10-15	28-35	40-45	-	-	-	-	±	+	±	4.9-4.1	5.3-4.6	0.40-0.70
<i>L. lactis</i>	18-20	37-40	50-52	-	-	-	+	±	-	±	4.1-3.9	3.7-3.6	1.50-1.60

\* Titratable acidity expressed as per cent lactic acid.

Defects due to bacteria of the coli-aerogenes group will be discussed in a following paper.

#### DISCUSSION

As in Tilsiter cheese (8), the normal ripening of Brick cheese apparently depends upon the development of a desirable bacterial flora with its members occurring in the proper sequence and proportion. The lactic acid starter bacteria which are added to the milk function chiefly in acid formation. Not only is the acid necessary to obtain the proper drainage of whey from the curd but also to prevent the subsequent occurrence of undesirable fermentations. These organisms persist in the cheese for varying periods during ripening, but probably do not play an important role in the curing.

Most of the ripening changes that are due to bacterial action depend upon the development of the desirable organisms from the natural flora of the milk. Probably the most important of these organisms is *Lactobacillus casei* to which has been ascribed much of the proteolysis and flavor production in other types of cheese. This organism always developed in substantial numbers in cheese made from raw milk, and if the acidity was favorable its growth was accompanied by the formation of a desirable body and flavor. However, when the milk was pasteurized, extensive development of *L. casei* was infrequent, and in its absence a product with a poor body and little or no flavor resulted. Thus, it may be said that the presence of *L. casei* is necessary for the normal ripening of Brick cheese. Other rod forms have been observed, but they appeared inconsistently and in relatively small numbers, hence they probably function little in the normal curing processes.

Of the other organisms present in the milk, *Str. bovis*, *Str. fecalis*, *Str. lactis*, *Str. thermophilus* and *Str. liquefaciens* were found at times in significant numbers. None of these except *Str. lactis* appeared consistently. *Str. fecalis* and *Str. liquefaciens* can utilize lactate as a food and can grow after the available sugar has disappeared. *Str. fecalis* is not proteolytic, hence probably functions only in flavor development. *Str. liquefaciens*, however, is strongly proteolytic and its action is accompanied by the formation of undesirable bitter flavors as shown in the few instances in which this organism developed in considerable numbers. It is believed that this species corresponds to Gorini's *acidoproteolytic* cocci and the gelatin liquefying cocci reported in the obligate ripening flora of Tilsiter (9) and Bel Paese (18) cheese. However, the effects of its growth in Brick cheese are such that doubt is cast upon its desirability. Anaerobic spore forming organisms invariably were present in the milk and developed when the acidity was low. They produced quantities of gas sufficient to split the cheese and caused an undesirable fermented flavor.

Completion of a study of this type makes it evident that certain phases of the work should be expanded and clarified. Closer examination of the physiological activities of the groups of organisms that appear during ripening should give a clearer understanding of their specific role in curing.



This would also involve the addition of these organisms to the cheese and following the course of their activity at intervals. It is possible that the addition of certain of the ripening organisms in small amounts with the starter might hasten the curing and make it possible to obtain a better product in a shorter time.

It should be emphasized here that most of the milk used in this investigation was of good quality. Perhaps if milk of poorer quality were used a different picture of the bacterial flora would be obtained. It is believed that the use of milk with a more varied and numerically greater initial flora than that employed in this study would demonstrate more clearly the role of lactate fermenting bacteria in the ripening processes, as well as the function of some of the other organisms.

#### SUMMARY

Examination of 1016 cultures isolated at different stages of ripening from 18 lots of Brick cheese yielded the following results:

1. *Str. lactis* was the predominant organism throughout the ripening of cheese in which it was used as part or all of the starter. Its numbers rose steadily to one to three billions per gram during the first one to four days, and declined again after three to four weeks. It also increased slowly in cheese to which it had not been added and reached numbers of several hundred millions per gram after one to two weeks.

2. When *Str. thermophilus* was used as the starter its numbers increased rapidly to a maximum of one to two billions per gram at twelve hours, then after one to two weeks they decreased sharply and the organisms seldom were found later in the ripening period.

3. Lactobacilli were found in the raw milk cheese after the first one or two weeks, but they seldom appeared in cheese made from pasteurized milk. *L. casei* occurred most frequently and in numbers up to ten to one hundred millions per gram; *L. brevis* was found less consistently than *L. casei* and usually in much smaller numbers; *L. lactis* appeared in a few of the samples after three to four weeks in insignificant numbers.

4. *Str. liquefaciens* was found in six of the lots of cheese after two to three weeks in numbers sometimes as high as 10,000,000 per gram. A few instances of bitter flavor probably were due to excessive numbers of this organism.

5. *Str. bovis* and *Str. fecalis* usually appeared in the cheese after one to two weeks, and ranged in numbers from a few thousands to as high as one hundred millions per gram. These organisms were most prevalent in cheese made from pasteurized milk.

6. Late gas formation by anaerobic spore forming bacteria occurred in all cheese in which the pH did not drop below 5.3 during the first three days of ripening. This condition was most pronounced in cheese made with *Str. thermophilus* starter by the washed-curd method from pasteurized milk.

The main function of the starter bacteria is a rapid and steady production of lactic acid during manufacture. The most important organism in bringing about the changes in body within the cheese during ripening is *L. casei*, and this organism also contributes to the flavor.

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# THE BABCOCK TEST; A REVIEW OF THE LITERATURE<sup>1</sup>

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The Babcock method for estimating the fat content in milk was one of the outstanding contributions to Dairy Science. When this method was 21 years old, Russell (181) stated that it was the only test used in the United States and Canada and was introduced into Argentina and South America and accepted in Australia and New Zealand before it was adopted in this country.

The Babcock test has served the dairy industry for more than 50 years without significant changes in technic and procedure. However, the American Dairy Science Association in annual meeting in 1938 recommended a study of this method with the objective of evaluating its accuracy. This recommendation was made because the dairy industry had questioned the progressiveness of the method in comparison with other important technological advances. A committee of the American Dairy Science Association is studying the procedure of the Babcock test and a detailed report will be forthcoming.

## VOLUMETRIC METHODS PRECEDING THE BABCOCK TEST

### *Europe*

The first record of a volumetric method of estimating fat in milk is that of Marchand (140, 141). His method was based on the assumptions that small amounts of sodium hydroxide had no serious effect on the fat, and that butterfat was soluble in ether and insoluble in water. He devised two test bottles called lactobutyrometers. One was of 10–11 mm. diameter throughout and divided into three parts. The lower one-third was calibrated for the milk, the middle for the ether, and the upper portion for the alcohol, the remaining volume of the tube being used for mixing. The upper part of the tube was calibrated in tenths and the very uppermost in hundredths of a cubic centimeter. The other instrument was somewhat different, having a lower bulb of 25 mm. diameter, 110 mm. in length and with a capacity of 53–54 cc. The detached graduated tube was calibrated, of 8 mm. diameter and had a capacity of 6 cc. The calibrated portion of the tube was graduated in tenths and arbitrarily referred to as degrees. The technique of Marchand's method consisted of adding 10 cc. of milk, followed by a drop or two of sodium hydroxide. Next the 10 cc. of ether were added, the tube stoppered and the contents well mixed. This was followed by the addition of 10 cc. of alcohol of specific gravity 86–90, and the contents carefully

<sup>1</sup> Published with the approval of the Director of the Vermont Agricultural Experiment Station.

mixed. The tube was placed vertically in a water bath at 40°<sup>2</sup> until it cooled to 30°. When the ether-fat layer had separated fully, the number of divisions on the graduated neck were read as degrees, and converted by a formula to weight of fat in grams. In a later paper (142) he gave more detail of how he arrived at the formula used. He stated that the amount of fat held below the ether-fat layer was 0.126 gm. per 10 cc. of milk, and the amount of ether and fat that separated was a constant. The formula he submitted was:

$$X = (N \times 2.33) + 12.6$$

X = value sought.

N = No. of degrees on the instrument.

2.3 = weight in grams of fat for each degree on the tube.

12.6 = grams of fat that remained in the aqueous phase per liter.

It is evident that Marchand's method is empirical. He cited the statement of Quevenne that milk contained an average of 34 grams of fat per liter.

Marchand's work stimulated more research on volumetric methods during the ensuing years. Bouchardat and Quevenne (34) reported comparisons of Marchand's with what was termed a chemical method but the results were divergent.

Schmidt (186) used five different lactobutyrometers, two were constructed according to Marchand's original instrument, one was made from a burette, and two were modified to provide more room for mixing the milk and reagents. He modified Marchand's reagents by using 3-5 drops of 5 per cent acetic acid instead of sodium hydroxide, used 90-92 per cent alcohol and read the tests at 20° instead of 40°. Forty estimations were made and compared with the gravimetric method of Tollens. The gravimetric method gave higher results. From these results he suggested another formula for the Marchand method so that the results would agree more closely with the gravimetric method. Applying this new formula to the 40 tests previously made, the two methods agreed within 0.1 per cent in 26 cases. He further stated that the Marchand lactobutyrometer did not give reliable results for adulterated milk.

Schmidt and Tollens (187) compared Marchand's with a gravimetric method on 30 samples of milk and they reported differences of 0.2 per cent and the greatest number did not agree within 0.1 per cent. They observed that separation of the fat was slower with alcohol of 86-90 per cent as compared to 91-93 per cent alcohol, and that the concentration of alcohol influenced greatly the estimations in milk with a high fat content. Using 92 per cent alcohol and reading the tests in a water bath at 20°, the results were compared with the gravimetric method and another formula was submitted. The application of this formula to the 30 samples of milk previously esti-

<sup>2</sup> All temperatures in this paper are in degrees Centigrade.

mated, resulted in three-fourths of the comparisons agreeing within 0.05 per cent. Krämer and Schulze (127) compared the Marchand lactobutyrometer with a gravimetric method and obtained results averaging 0.32 per cent lower with the former method.

Tollens and Grote (215) compared Marchand's with a gravimetric procedure and concluded that the volumetric method showed reliable results for all practical purposes; however, their limited data indicated the volumetric yielded lower results than the gravimetric method. They emphasized the importance of shaking the contents vigorously until the ether and milk formed a uniform mixture for the moment. The tests were kept in a water bath at 40° for 5–10 minutes and the ether-fat layer read and the per cent fat obtained from a prepared table. The reagents and milk were placed in the lactobutyrometer with pipettes. No marked differences were revealed when the tests were read at 20° and at 40°.

Schmoeger (188) tested 110 milks with the gravimetric and the Marchand method using the butyrometer modified by Tollens and found that the latter gave results 0.1 per cent lower. Using 92 per cent alcohol, the volumetric test yielded the highest results, but both 90 and 92 per cent alcohol contributed results lower than the gravimetric method. He stated that the modified method submitted by Schmidt and Tollens (187) gave more accurate results when 3 drops of 15 per cent potassium hydroxide were used and separation of the ether took place at 20°, otherwise, their method gave results 0.2 per cent too low.

Dietzsch (53) compared Marchand's with Soxhlet's aerometric method and reported agreement within 0.5–0.1 per cent on numerous samples. He recommended leaving the butyrometers in the water bath at 38° for 10–15 minutes, and emphasized the importance of mixing reagents and milk, otherwise, reliable results were not obtained. Marchand's method was used in the Anglo-Swiss condensed milk laboratory. Peter (169) used essentially the same procedure and obtained close agreement on the majority of 84 comparisons with a gravimetric procedure. Sjostrom (201) modified Marchand's method for milk containing 1.78 to 4.31 per cent fat.

Liebermann (133) contributed a method whereby 50 cc. of milk were placed in a large cylinder and 5 cc. of sodium hydroxide and 50 cc. of ether were added. The contents were shaken from one to two minutes to a homogenous yellow color. After allowing the contents to settle for 10–15 minutes, a 20-cc. aliquot was evaporated on a water bath at 40–50°, then dried for 15 minutes at 100–105°. The air-free fat was measured in a carefully calibrated flask and converted to per cent by weight, assuming a constant value for the specific gravity of butterfat. Close agreement was obtained with Hoppe-Seyler's gravimetric procedure on five samples of milk.

Cronander (44) constructed a test bottle of 200–250 cc. with two glass tubes, one calibrated and the other to carry hot water to force fat into the

graduated neck, 10 cc. of potassium hydroxide were added to 100 cc. of milk and thoroughly shaken with 30 cc. of ether. The flasks were allowed to stand quietly for 30 minutes, then placed in a water bath at 60–65° for 1–1½ hrs. and at 80° for 30 minutes. The fat was forced into the measuring tube and the estimated volume converted to per cent from a table. Close agreement was obtained with Soxhlet's method.

Schmid (185) contributed a method that involved the use of a 50-cc. tube, graduated in tenths. Ten cc. of milk or 5 cc. of cream were measured into this tube and 10 cc. of concentrated hydrochloric acid added and the mixture heated until a dark brown color appeared. The contents were cooled and 30 cc. of ether added and mixed. When the separated ethereal layer was clear, 10 cc. was pipetted into a tared dish and evaporated over a water bath at 100°. The results checked within 0.1 per cent of the gravimetric procedure and required about 15 minutes. The name of the Helvetia Milk Condensing Company, Highland, Illinois, appeared at the end of this paper. Stokes (211) modified this method slightly and reported excellent results and Hill (96) reported satisfactory results with a slight modification of Schmid's (185) method. Léze (132) submitted a similar method whereby milk was heated to near boiling with two volumes of hydrochloric acid, then saturated with ammonium hydroxide and the contents became clear as the fat rose to the surface. He suggested the use of the centrifuge to speed the separation of the fat.

The volumetric methods cited up to this point have been empirical, rather time-consuming and cumbersome. De Laval (54) in 1885 patented a method (55, 56) for estimating the fat content of milk. He was the first to describe the use of the centrifuge in a volumetric fat determination. He used a disc that revolved in place of the bowl in his centrifugal milk separator. The original method called for the use of an equal amount of milk and a mixture of 20 parts concentrated acetic and one part of sulphuric acid. Ten cc. of milk at 15° were placed in a boiling tube containing 10 cc. of the acid mixture, heated to 80° for 15–18 minutes with occasional agitation. Next the boiling tubes were placed in water at 60° until the contents reached this temperature. The tubes were well mixed and some of the contents poured into small cups; next the small graduated test bottles were filled by pushing them into these cups and the excess flowed through the top into a suitable receptacle. The centrifuge was heated with hot water or steam to keep the temperature above 50° and whirled at 6,000 r.p.m. for 3–5 minutes. The test bottles were taken from the centrifuge and read while the small hole at the top was closed with the finger. These bottles were empirically calibrated in 0.1 per cent divisions to check with the gravimetric procedure. Tests could be read to 0.02 per cent accuracy. De Laval named this the lactocrite test. Engling and Klenze (66) stated that the lactocrite procedure was cheaper and checked closely with Soxhlet's method. Sebelien (192) re-

ported close agreement between the lactocrite and a gravimetric procedure; similar results were reported by Soxhlet (205), while Frahm (82) stated that the lactocrite required more expensive equipment. Nilson (160) supplied a table of corrections so that the method was more nearly accurate on low testing milk. Blyth (33) and others (128, 189) reported close agreement between the lactocrite and the gravimetric procedure, while Soxhlet (205) reported similar results with his aerometric method. Faber (67) reported close agreement between the lactocrite and a gravimetric method and also lauded the simplicity of the former. Gerber's method (85, 86) apparently had not received extended experimental attention beyond a description of the butyrometer and the reagents used and did not assume its present form until 1895 (22). Barthel (22) has described the lactocrite method in detail. Oven (163) has contributed a review of the literature of some of the methods used in Europe.

#### *United States*

While European research workers were seeking to develop rapid methods of estimating fat in milk, chemists in America were engaged in similar activities.

Short's method (197) consisted of saponifying the fat in 20 cc. of milk with 10 cc. of an alkali that contained 250 grams of sodium hydroxide and 300 grams of potassium hydroxide dissolved in 1809 grams of water. The saponification process continued for two hours. The insoluble fatty acids were obtained by boiling one hour with 10 cc. of an acid mixture consisting of equal parts of sulphuric and acetic acids. Tubes were filled within one inch of the neck and allowed to stand in hot water without boiling. The fat column was estimated in millimeters and from the weight of the milk and the specific gravity of the insoluble fatty acids, the per cent of fat was calculated. Short (197) submitted analysis on a number of samples of milk and the results were approximately 0.04 higher than the gravimetric method. Results obtained by his assistants did not agree so closely. Manns (139) compared Short's method with a gravimetric procedure and the greatest variation was 0.23 per cent. Farrington (70) on a few samples found that Short's method yielded results that were too high and similar results were reported by Frear and Holter (83). Myers and Magruder (153) reported close agreement between Short's, the lactocrite and the lactobutyrometric methods.

Parsons (165) criticized Short's method for not being accurate for skim-milk and buttermilk containing less than 0.50 per cent fat. He developed a method whereby 10 cc. of cream were measured into a 250-cc. flask with the addition of 10 cc. of 50 per cent sodium hydroxide, plus 5 cc. of 95 per cent alcohol containing one ounce of castile soap per gallon, and finally 50 cc. of gasoline. The flask was shaken vigorously for a few seconds and five or six times at equal intervals within the next half hour. If the fat-gasoline



layer did not separate, repeated additions of the alcoholic soap solution were made until separation occurred. Twenty-five cc. of the fat-gasoline material were placed in a flask to evaporate, and the residue dried at 245–255° for 30 minutes. The fat was collected in a calibrated tube cooled to the first evidence of solidification, held in the hand for a moment and estimated to the uppermost meniscus; this reading was converted to per cent fat from a table. Close agreement with the gravimetric and with Soxhlet's method was obtained on milk, cream, skimmilk and buttermilk. It was estimated that 40 analyses could be made daily. Professor E. H. Farrington collaborated in this work. Collier (40) experienced difficulty with this method as originally described, so he used 15 cc. of the alkali and 10 cc. of the alcoholic soap solution. Unskilled workers obtained results that averaged 0.03 per cent from the gravimetric method.

Failyer and Willard's (69) method is similar to that of Schmid (185). They used hydrochloric acid and milk in a tube similar to that devised by Short (197), except the graduated neck was of smaller diameter. The test bottle was 10 inches long with the graduated portion being 3½ inches long and 4–5 mm. in diameter. Ten cc. of milk were added to the bottle and heated to near boiling over a flame with 8 cc. of acid carefully added. The contents were heated cautiously for about five minutes with careful agitation and cooled. Next 15–20 cc. of gasoline were added and the bottle gently agitated to dissolve the fat. The bottles were placed in an inclined position in a boiling water bath and air blown into the tube to evaporate the gasoline. Water was added to the base of the neck and the bottle whirled (time not stated) a few times and allowed to stand in a boiling water bath for a few minutes. Boiling water was added to bring the fat into the graduated neck. Readings were made in the water bath. Single analyses require about 25 minutes. It was suggested that the test bottles could be graduated in fractions of a cubic centimeter with 0.87 as the specific gravity of the fat. The results checked closely with a gravimetric procedure used by Babcock.

Cochran (42) contributed a method whereby milk was heated at about 100°, for 10–15 minutes in a special flask with 5 cc. of a mixture of equal parts of acetic and sulphuric acid and until the contents assume a coffee color. The flasks were cooled and 4 cc. of ether added; boiling was continued for 10–15 minutes. The fat was poured into a flask with a side tube and boiling water added to bring the fat into the graduated neck. Estimations were made at 65.5° and converted to per cent with the aid of a specially prepared table. Caldwell (36) reported a preference for this method over those of Parsons (165), Short (197) and Failyer and Willard (69), while Magruder (137) obtained variable results with this method and Farrington (70) and Frear and Holter (83) reported close agreement on a few samples. This method was standardized to agree with the Adams gravimetric method and was used in about 50 creameries in southwestern Pennsylvania. It was claimed that 60 determinations could be made in 2–3 hours.

Patrick (166) released details of the Iowa test in June, 1889, through the *Farm Journal Homestead*. This test utilized an acid mixture to dissolve the solids-not-fat, consisting of 9 volumes of 90 per cent acetic acid, 5 volumes of concentrated sulphuric and 2 volumes of concentrated hydrochloric acid and the solution saturated with sodium sulphate. The test bottles had a bulb with a capacity of 21 cc. and two-thirds the distance from the bottom a small orifice was located, 1 to  $1\frac{1}{2}$  mm. wide, and closed with a rubber band. The graduated neck was 6 to  $6\frac{1}{2}$  cm. long, being carefully calibrated to 1 cc. capacity. The tube above the neck was  $14\frac{1}{2}$  cm. long. A 10.8-cc. pipette was used and later changed to hold 10.4 cc. to allow the method to check more closely with the gravimetric procedure. After adding the acid, the bottles were placed in a hot sand bath and allowed to boil slowly for 4–6 minutes. The fat rose and the bottles were again allowed to boil gently for 5–8 minutes to clarify the fat. The tests could be read directly from the sand bath, but more accurate results were obtained by immersing the bottles in water at  $140^{\circ}$  for 10–15 minutes and for more exact results immersion for 30 minutes was recommended. The fat column was estimated from the extreme lower to the extreme upper part. Farrington (70, 71) reported close agreement with the gravimetric method on a few samples using this method. Patterson (168) found that the methods of Beimling (5) and Patrick (166) agreed closely with the gravimetric method.

Leffmann and Beam (130, 131) submitted specifications for a test that they claimed was devised in 1889. Test bottles of 30 cc. capacity with calibrated necks containing divisions equivalent to 0.1 per cent fat were used. The test was conducted by measuring 15 cc. of milk into the bottle followed by 3 cc. of a mixture of equal parts of amyl alcohol and "strong" hydrochloric acid, mixed well, filled nearly to the neck with concentrated sulphuric acid and further mixed with a rotary motion. The bottles were filled to about the zero point with diluted sulphuric acid, and whirled in a centrifuge for 1–2 minutes. The estimations were made with dividers, allowance being made for the meniscus. Results agreed within 0.1 per cent of the Adams method.

A test was described by the Vermont Agricultural Experiment Station (5) that involved the use of the same kinds of reagents as the methods of Leffmann and Beam (130, 131) and of Gerber (85); but the glassware in the Gerber method was different from the other two. The centrifuge was patented by H. F. Beimling, an Austrian chemist living in Philadelphia, Pennsylvania, and was sold by the Creamery Package Manufacturing Company (43). About 200 of these centrifuges were sold from 1890–1902. Special glassware was devised by the Vermont Station (5). It was stated that 75 per cent of the results agreed within 0.1 per cent of the Adams gravimetric method. Magruder (137) expressed a high regard for what he called the Beimling method (5). Russell (181) stated that Babcock devel-

oped a method similar to Soxhlet's aerometric procedure whereby the milk was made strongly alkaline and the fat was extracted with ether. The ether was evaporated and the fat estimated in bottles similar to those used in the regular Babcock test and without transferring the contents. This method was discarded because the results did not check in all instances with the gravimetric method.

Thus, the literature reveals a background of much research on volumetric methods of estimating fat before Babcock made his contribution to dairy science.

#### STANDARDS OF ACCURACY FOR BABCOCK GLASSWARE

When Babcock (10, 11) contributed his test, there were two volumetric dimensions in use, namely, the Mohr cubic centimeter of one gram of water at 15°, and the true centimeter of water at 4°. The use of both created considerable confusion among manufacturers of glassware and state officials. Holland (103) recognized the difficulties involving the use of two volumetric units and pioneered in obtaining the adoption of one fundamental standard for calibrating Babcock glassware. He wrote to three prominent manufacturers and found that the Wagner Glass Works used one cubic centimeter as equal to 13.59 grams of mercury at 15.5°, which is the Mohr unit and the Kimball Glass Company used the true cubic centimeter and calibrated with mercury, specific gravity 13.5463 at 20°. The Emil Greiner Company (64) reported to Holland (103) that they made the first test bottles for Babcock who told them to graduate the neck of 2 cc. capacity into 50 parts with each 5 parts to represent 1 per cent butterfat and they used the Mohr unit. Babcock (12) stated that the neck of the 10 per cent milk test bottle represented a volume of 2 cc. that is equivalent to 2 grams of water or 27.18 grams of mercury, specific gravity 13.59. The temperature was not stated but it was assumed that 15.5° was intended.

Holland (103) realized the necessity of having one absolute unit of volume so that test bottles and pipettes could be calibrated on a scientific basis. Consequently he had a recommendation made to the Bureau of Standards at Washington, D. C., which was adopted, that the true cubic centimeter of water at 4° and weighing 0.998877 be the unit of graduation for all Babcock glassware. In addition he made recommendations for methods of calibrating and for standards of accuracy of glassware, approved by Babcock, which appeared in the Association of Official Agricultural Chemists (8) (A.O.A.C.) in 1909 and have remained unchanged, with minor exceptions, to the present time. In this connection it should be stated that Professor Hunziker (116) rendered monumental service to the dairy industry from 1910 to 1926 in the capacity of Chairman of the Committee on Official Methods of Testing Milk and Cream for Butterfat in the Official Dairy Instructors Association (O.D.I.A.), now the American Dairy Science Association (A.D.S.A.). This committee called a meeting in Washington, D. C., in 1911 with members of the O.D.I.A., the U. S. Bureau of Dairying, the

U. S. Bureau of Standards and manufacturers of glassware. Standard specifications for Babcock glassware were formulated and adopted and pronounced official and published by the A.O.A.C. (8) and by Standard Methods of Milk Analysis (210) (S.M.M.A.). Hunziker's committee also drafted a standard procedure for the Babcock test that has been universally adopted with or without minor modifications and was published by the A.D.S.A. (3) in 1917. These refinements in glassware and procedures marked a distinct milestone of progress since 1902 when the A.O.A.C. (8) stated that due to the wide publication of Babcock's method it was not deemed advisable to publish the detailed procedure. Andrews (4) advocated state control and supervision of Babcock testing equipment and procedures.

In view of the fact that this discussion will involve the manipulation of the various technics of the test, it was thought advisable to state the present procedure for the Babcock test so that persons in countries where it is not common may more easily follow the context. The procedure for milk in the A.O.A.C. (8) in 1940 is outlined as follows:

1. Transfer 18 grams of milk with a 17.6-cc. pipette to the test bottle, blowing out the last drops after free outflow has ceased.

2. Add 17.5-cc. sulphuric acid at 15–20° (sp. gr. 1.82–1.83 at 20°) preferably not all at one time, pouring it down the side of the bottle neck to wash all traces of milk into the bottle.

3. Shake bottles until the curd has disappeared, place them in the centrifuge and counterbalance each bottle.

4. After the proper speed has been attained, whirl for five minutes. Add soft water at 60° or above to fill the bulb of the bottle. Whirl two minutes. Add hot water until the liquid fat approaches the top graduation on the neck. Whirl one minute longer at 55–60°.

5. Immerse the bottles in a water bath maintained at 55–60° to the level of the top of the fat column until the column is in equilibrium and the lower fat surface has assumed final form.

6. Remove the bottle, wipe it, and measure the fat column with calipers from its lower surface to the highest point of the meniscus. The fat column at this time should be translucent, of golden yellow or amber color, and free from suspended particles.

The procedure for cream is essentially the same in principle, except that it is weighed instead of measured by volume.

The discussion to follow is a summary of the technics that have been used in the sequence of conducting the Babcock test.

#### TECHNICS

##### *Preparation and Care of the Sample*

The A.O.A.C. (8) in 1908, 1916, 1920 and the A.D.S.A. (3) in 1917 emphasized the importance of careful mixing until a homogeneous sample

was obtained. Since 1925 the A.O.A.C. (8) specified in addition that the sample should be tempered to 20°, and if lumps of cream prevailed, heat to 38° and cool to 20° before pipetting; similar recommendations were made by S.M.M.A. (210) in 1928, while the A.D.S.A. (3) in 1917 specified that abnormal samples should be heated to 38–49°, mixed thoroughly and pipetted at once.

Babcock (10, 11) emphasized the importance of securing a representative sample by pouring from one container to another at least three or four times, and milk with a cream layer should be given this treatment until no lumps appeared on the surface, but he cautioned against excessive agitation. Farrington (72) and others (35, 52, 63, 80, 84, 91, 135, 143, 159, 206, 234) recommended pouring milk from one vessel to another at least three or four times. Crowe and Davis (45) recommended slow pouring at 16–21° to avoid excessive incorporation of air, while Ball (21) supplemented the pouring process by shaking the samples at 18–24° and if necessary, heating to disperse the fat and cooling to this temperature before sampling. Smith (202) gently rotated the sample and poured it through a fine wire sieve. Dahlberg (46) specified heating and sampling preserved composite milks at 38–43° because they could not be cooled at 21° without some destabilization of the fat; later Dahlberg and Powers (49) suggested sampling at 32.2–37.7°. Hunziker (115) and others (157, 212) stated that partially churned milks and those that had a dried cream layer should be heated slowly at 29–43° and mixed carefully to avoid further injury to the emulsion. Kerr (124) stated that milk should be pipetted immediately after shaking to obtain an accurate sample. Caldwell and Herreid (37) sampled milks at 4.4, 21.1, 37.8 and 60° and found that those sampled at 60° tested 0.08 per cent lower than at 4.4°, while at 37.8° the test was 0.04 per cent lower than at 4.4°. Liverance (135) and others (35, 52, 91, 113, 122, 164, 212) suggested sampling temperatures ranging from 10–27°.

Composite sampling was conceived of as the dairy industry grew in commercial prominence with demands for increased efficiency through the use of labor saving devices. Patrick (167) first proposed the use of composite samples in this country. Babcock (13) suggested that a satisfactory composite could be obtained by using a test bottle twice the usual size for each patron and measuring into this bottle 5 cc. of milk daily for seven days. Shutt (199) suggested a similar procedure by taking 2.93 cc. of milk daily for six days.

Heinreich (93) reported that milk could be measured into test bottles and left for weeks without affecting the accuracy of the results. Furthermore he reported that finished tests could be kept for a considerable time and reread by placing in warm water and centrifuging. Hills (99) used this procedure on tests that were kept for three years and exposed to temperatures from –18 to 38°. Each year the tests were heated and read. Two

years did not seriously affect the results, but after three years the tests were of no value.

Kent (123) compared the aliquot and dipper methods for preserved composite samples with the dipper giving the lowest results, while Potts (173) obtained only slight differences. Tracy and Tuckey (218) concluded that aliquot sampling was not necessary but that inaccurate sampling may lead to erroneous results. Marquardt and Durham (144) stated that stirring milk in the weigh can was not necessary, while Bailey (18, 19, 20) reported that agitation was essential. Webster (224) reported significant differences in the fat content of milk from different parts of the weigh can. Powers (174) concluded from an extensive survey that there was a tendency for non-agitated samples to test higher, but that in general, sampling was affected by other conditions in individual plants.

The care of preserved composite samples received early consideration by Babcock (15) who recommended keeping them in a cool place. The A.D.S.A. (3) in 1917 and in 1922 stated that composite samples should be kept cool and be the product of not more than one week of milk deliveries. Sanmann and Overmann (182, 183) submitted data to show that composite samples should be stored at 10° or lower.

With the advent of composite sampling, a number of preservatives received experimental attention. Patrick (167) studied a number of preservatives including borates, salicylates and bichloride of mercury. He concluded that bichloride of mercury in concentrations of 10–15 grains to 200–400 cc. of milk was the most satisfactory. Jackson (119) reported no difference in results by varying the concentration from 0.5 to 1.5 grams bichloride of mercury per 150–300 cc. of milk. Campbell *et al.* (38) and others (65, 115, 146, 147, 152, 182, 183, 220, 225) reported the use of this preservative. Grélot (88) stated that small amounts of ammonium chloride expedited the solution of bichloride of mercury.

Farrington (72) used a preservative consisting of a mixture of 2 ounces of bichloride of mercury, 2 ounces of fine "salt," 8 ounces of finely ground borax and 1.5 drams of aniline red. He used 15–20 grains of this mixture for each sample jar.

Alén (1) preserved milk by adding 0.5 grams of potassium dichromate to 250–500 cc. of milk and stated his intention of having this method patented. Babcock (15) and others (25, 87, 88, 102, 134, 153, 158, 178, 195, 199, 230, 234, 235, 236) used this preservative in varying concentrations.

Rideal (176) stated that one part of formalin to 10,000 parts of milk kept it fresh for 7 days. Thompson (214) preferred formalin to boric acid, borax, salicylic or benzoic acid. McConnell (154) reported formalin equal to potassium dichromate, but preferred the latter because it colored the milk. Lindet (134) stated that formalin interfered with fat determinations by Gerber's method, while Bevan (31) and Hills (97) added increasing amounts

of this preservative to milk and obtained a parallel increase in total solids. Hills (97) experimented with a number of preservatives and concluded that the most useful were mercuric chloride, a mixture of 10 parts mercuric chloride with 50 parts of borax, potassium chromate and the bichromate, sodium sulphite and bisulphite, copper ammonium sulphate, sodium salicylate and formaldehyde.

Other preservatives used were copper ammonium sulphate by Windisch (230), who has also contributed an excellent review of the literature on preserving milk for chemical analysis; Farrington (73), who used a teaspoonful of 98 per cent sodium hydroxide to each sample; Ronneberg (178), who used potassium permanganate and Neumann (158), who used ammonium hydroxide and ammonium carbonate.

### *Cream*

The sampling of cream for testing received some experimental attention because of its viscous nature. The A.O.A.C. (8), from 1908 to 1916 inclusive, gave no detailed directions for sampling, but referred to the various editions on testing published by Van Slyke (222) and by Farrington and Woll (77).

Van Slyke (222) stated in 1913 that thick cream of low temperature should be warmed to not above 30–35°, poured carefully and quickly sampled, while if the cream was completely separated it should be heated to 40–43°, continuously shaken and cooled to 15.5° and quickly weighed. Farrington's and Woll's (79, 80) recommendations applied chiefly to the sampling of cream from individual patrons.

In 1917 and in 1922 the A.D.S.A. (3) stated that if the cream was in good condition, the samples could be mixed by shaking, pouring or stirring, but if very thick they should be warmed to 30° and then mixed. If lumps of butter were present, or if a large number of samples were to be tested the cream should be heated to 38–50° in a water bath, being careful to avoid "oiling off." It was also recommended that the samples be kept cool and stored in non-adsorptive air-tight containers. The A.O.A.C. (8) in 1925 and S.M.M.A. (210) in 1934 published this procedure that has appeared in subsequent editions.

Babcock (10) stated that cream required thorough mixing and if sour and exposed to the air until the surface had dried, proper sampling was difficult. Hunziker (115) stated that normal fresh cream could be sampled accurately by rotating or by careful pouring, but thick cream should be heated to 29–32°, poured gently a few times and weighed immediately, while cream in poor physical condition should be heated to 43° or above, and continuously shaken while cooling to 16° or lower before weighing. Hills (100) suggested that lumpy cream be passed through a small sieve and mixed by pouring from one cup to another. Webster (223) and others (2, 20, 91, 113, 122, 143) emphasized care in the preparation of the cream sample.

*Size of Charge*

*Milk.* In 1902 the A.O.A.C. (8) referred to Wiley (228) who stated that a 17.6-cc. pipette delivered a volume that varied but slightly from the desired weight of 18 grams. In 1908 the A.O.A.C. (8) specified a 17.6-cc. pipette, while in 1916 this organization stated the dimensions of the pipette as specified by the United States Bureau of Standards (221) and by the A.D.S.A. (3) in 1917. This pipette was calibrated to deliver 17.6 cc. of water at 20° in 5 to 8 seconds and since 1925 the A.O.A.C. (8) has specified the pipette to contain 17.6 cc. of water at 20° when the bottom of the meniscus coincided with the line on the draw tube. Since 1923 S.M.M.A. (210) has also published these specifications. Furthermore, the A.D.S.A. (3) in 1921 recommended that the discharge stem of the pipette be made to slip into the test bottle, and thus eliminate the awkward method of holding the tip of the pipette in a slanting position against the neck of the test bottle. It was also suggested that the last drop of milk, after free outflow ceased, should be blown into the test bottle. S.M.M.A. (210) in 1923 and the A.O.A.C. (8) in 1925 incorporated this recommendation.

Babcock (10, 11, 12) stated that when the pipette was filled so that the upper surface of milk coincided with the mark on the draw tube it should contain 17.6 cc., but would deliver slightly less than 17.5 cc. and 17.44 cc. of milk would be equivalent to 18 grams, assuming an average specific gravity of 1.032.

Bailey (17), Dahlberg (50) and Hoyt (111) used pipettes calibrated to deliver 17.6 cc. of water at 20° C. Bailey (17) reported a delivery of 17.924 grams of milk at 21°. He determined the influence of temperature and at 21° the average delivery was 17.937 and at 46° was 17.814 grams of milk. The pipettes were allowed to drain 2 to 3 seconds and the tips were touched against the inside of the necks of the bottles. Dahlberg (50) weighed the deliveries of six samples at 21.1° and at 48.8° and obtained weights of 17.9680 and 17.8425 grams, respectively; the estimated fat content on each were 4.81 and 4.82 per cent. Bartlett (26) and others (12, 35, 52, 80, 84, 91, 92, 113, 122, 135, 143, 159, 206, 212, 231) specified the use of 17.6 cc. of milk, but the intent of calibration of the pipette was not always certain; with few exceptions they recommended blowing the last drops from the pipette. Hoyt (111) obtained an average delivery of 18.015 grams of milk from 36 determinations; the temperature of the milk was not stated, but the pipette was calibrated to deliver 17.6 cc. of water at 20° in 5 to 8 seconds. Bearce (29) has submitted data to show the weight-volume relationship of milk and cream at practical temperature ranges.

*Cream.* The A.O.A.C. (8) in 1908 stated that cream should be weighed and in 1916 and 1920 this organization specified the use of 9 and 18 grams, depending on the fat content. In 1917 the A.D.S.A. (3) mentioned either



9 or 18 grams, using standard cream test scales set level with a sensitivity reciprocal of 30 mgs. and the weights should preferably be stamped for accuracy by some official organization. In addition to these requirements, the A.O.A.C. (8) since 1925 has specified that the scales be protected from air currents and that the weights consist of material capable of resisting corrosion and be of the low squat type with rounded edges.

Babcock (10, 11) suggested dividing the contents of the 17.6 cc. pipette, including rinsings between three milk test bottles, but recognized the errors involved that could be eliminated by weighing the cream. Eckles (60) made similar suggestions. Winton (232) devised a pipette for cream that delivered 6 grams; 12 cc. of water were added and the remainder of the procedure was the same as for milk. Jones (121) presented data to show the fallacy of measuring cream by volume for the Babcock test. Spillman (207) measured cream by volume and devised a table of corrections for variable contents of fat. Webster (223), Farrington and Woll (80) and others (21, 35, 45, 84, 91, 113, 115, 122, 135, 143, 159, 212, 216) used 9- or 18-gram charges and there was some division of opinion as to the merits of the 9- and 18-gram bottle.

### *Type of Bottle*

*Milk.* The bottle designed by Babcock (10, 11) was the same shape as that used by Short (197), except it was smaller and of heavier glass. Babcock's bottle was graduated from 0-10 in 0.2 per cent divisions. This same type of bottle was illustrated by Wiley (228) in 1897 as official, and presumably this same bottle was listed as official by the A.O.A.C. (8) in 1908. In 1909 the A.O.A.C. (8) stated that the capacity of each per cent should be 0.2 cc. as calibrated with clean dry mercury at 20°, and the limit of error should be the smallest graduation and not to exceed 0.5 per cent. The O.D.I.A. (161) in 1911 adopted specifications that were restated in detail by the A.D.S.A. (3) in 1917 and 1922 and are summarized as follows: The bottle should have a total height of 150-165 mm. with a bulb capacity of at least 45 cc. and if cylindrical, the body must have a diameter of 34-36 mm., and if conical, the base must have a diameter of 31-33 and a maximum of 35-37 mm. The graduated portion of the neck should be not less than 63.5 mm. long with calibrations in whole, half and tenths from 0.0 to 8.0 per cent. The 0.1 per cent graduations must not be less than 3 mm. long, the 0.5 per cent not less than 4 mm. long and must project 1 mm. to the left, while the whole per cent markings must extend at least half way around the neck to the right and project at least 2 mm. to the left of the tenth per cent graduations. The cylindrical part of the neck must extend 5 mm. below the lowest and above the highest graduated mark and the top must be flared at least 10 mm. wide. The volume of each whole per cent should be 0.2 cc. and the maximum error must not exceed the smallest unit of graduation. These detailed specifications appeared in the A.O.A.C. (8) and S.M.M.A. (210) in 1925 and 1928, respectively.

Mitchell and Walker (148) described a test bottle and a centrifuge whereby water could be added to the bottle while the machine was in motion. This bottle had in addition to the Babcock, a second funnel-shaped neck for receiving water from a spindle. The water was forced outward by the centrifuge and caught by the cone-shaped necks of the bottles. The lower end of this water neck was reduced in size to prevent fat from rising into it. It was claimed that churning of the milk was avoided because the acid flowed down the outer wall of the bottle, thus avoiding immediate mixing. Bartlett (26) devised a milk test bottle with a graduated body that eliminated the need for an acid measure. Whitman (227) designed a bottle for testing as little as 2.5 cc. of milk. The body of this bottle was calibrated for the milk charge and to fit trunnion carriers in a small centrifuge. A ground glass graduated tube was placed into the shoulder of the bottle to collect fat. The results were claimed to be identical to those obtained with the Babcock test bottle. Manchester (138) presented data to show that the length of the graduated neck on the Babcock test bottle was a factor in obtaining accurate results. He reported the errors introduced when the length of graduations in a 0-10 per cent bottle were varied as follows: 60-65 mm. 0.1 per cent, 45-50 mm. about 0.2 per cent, and 22 mm. about 0.55 per cent. Bottles with graduation lengths of 80 mm. were reported as the most accurate.

*Cream.* The invention of the Babcock test naturally led to its application to cream and other milk products. Winton (234) developed a test bottle that appears to have been the pattern used for the present cream bottle. The committee in the O.D.I.A. (161), previously referred to, in 1911 gave detailed specifications for the 50 per cent 9-gram cream bottle in which the total length, length of neck, capacity and dimensions of the body and general methods of marking were essentially the same as for the whole milk bottle; the essential difference being in the size and capacity of the graduated neck. The same specifications applied to the 9 inch bottle, except the total height was 210-225 mm. These bottle specifications were restated by the A.D.S.A. (3) in 1917 and in 1922 together with dimensions for the 18-gram 50 per cent bottle, all of which appeared in the A.O.A.C. (8) in 1925 and in S.M.M.A. (210) in 1934. These detailed specifications were inspired by the recommendations of Huuziker *et al.* (117) who illustrated five different types of 6-inch and the same number of 9-inch bottles. They condemned both 50 per cent 18-gram 6-inch bottles, one with a bulb in the graduated neck and the other with a straight neck, because the large diameter of the necks made accurate reading very difficult. The 30 per cent 18-gram 6-inch bottle was not recommended because of its limited range.

Bartlett (23, 24) described a test bottle with a neck sufficiently long to estimate cream up to 25 per cent fat. He devised a second bottle for cream containing up to 35 per cent fat, with a removable neck that required a larger centrifuge. Later (26) he contributed a bottle for cream with a smaller

body that was calibrated for the proper amount of acid. Bartlett (26) also submitted a bottle to estimate the fat in butter using an 18-gram sample. Farrington (75) contributed an 18-gram 6-inch bottle with a body of 45 cc. capacity and with a neck calibrated from 0-30 in 0.5 per cent divisions. Bebee (30) used an 18-gram 50 per cent 9-inch bottle, with the zero mark at the top of the neck. Holter (107) devised a test bottle with a large graduated neck for butter and which could be used for cream of high fat content.

*Amount, Strength and Temperature of the Sulphuric Acid.*

**Milk.** The amount of acid specified by all official organizations was 17.5 cc. of specific gravity 1.82-1.83. Since 1925 the A.O.A.C. (8) and since 1928 S.M.M.A. (210) stated the specific gravity of the acid at 20° and the temperature of adding acid to 15-20°, preferably not to be added at one time. The A.D.S.A. (3) in 1917 and 1922 specified the temperature of the acid at 10-21°. The acid was designated, with few exceptions, to be measured with a graduate or a Swedish acid bottle that would deliver 17.5 cc. The A.O.A.C. (8) in 1916 and 1920 specified that the error of volume for acid measures should not exceed 0.2 cc.

Babcock (10) recommended 17-18 cc. of acid with a specific gravity of 1.82-1.83. He (13) later recommended 17.5 cc. of 1.82-1.83 specific gravity, and with few exceptions, these specifications for acid have been followed by others (21, 22, 35, 45, 63, 74, 84, 113, 116, 143, 159, 212, 234).

Bartlett (26) recommended 20 cc. of acid of 1.82-1.825 specific gravity and advised adding hot water to the base of the bottle neck immediately after mixing acid and milk; this was a slight modification of the Babcock test.

Bailey (17) used varying amounts of acid of 1.80, 1.81, 1.82, and 1.83 specific gravity with acid and milk at 21°. The acid of low and smaller amounts of high specific gravity resulted in practically colorless fat columns that averaged 0.05 per cent lower than when 17.5 cc. of the stronger acid was used.

Petersen (170) found that when sulphuric acid was contaminated with butterfat, lard and the oils of cottonseed, olive, corn, cod, sperm and of mineral, that the fat estimations of milk were increased. Later (171) he prepared a stable emulsion of sulphuric acid, specific gravity 1.83, fat saturated benzine and a water soap solution that also increased the fat readings. This emulsion could not be separated by whirling in an 18-inch centrifuge at 1,000 r.p.m.<sup>3</sup>

Babcock (10, 11, 13) did not mention a specific temperature for milk and acid. Bailey (17) made estimations with acid and milk at 10°, 21° and 32°, using acid of specific gravity 1.83, and found equally good results if the amount of acid was varied inversely with the temperature. He recommended the lower temperature to avoid charring the fat. White (226) ob-

<sup>3</sup> Stated 10,000 r.p.m., but this is an error.

tained the most accurate results when sufficient acid at  $15.5^{\circ}$  was used to give an almost colorless fat column. Farrington (74) stated that acid of 1.82 specific gravity should be used with milk at  $16-21^{\circ}$  and if the acid is stronger, cool to a lower temperature. Bartlett (26) and others (35, 45, 74, 122, 159, 202, 206, 212) stated that milk and acid should be tempered to  $16-21^{\circ}$ , while Farrington and Woll (80) and others (91, 113, 116, 143) suggested temperatures ranging from  $13-27^{\circ}$ .

*Cream.* The O.D.I.A. (161) described three methods of adding sulphuric acid that was published by the A.D.S.A. (3). In the first method sufficient sulphuric acid was added until the mixture resembled the color of coffee, containing cream. The amount needed depended on the temperature of acid and cream and the fat content of the cream, but varied from 8–12 cc. for a 9-gram and 14–17 cc. for an 18-gram sample. The second method was only applicable to the 9-gram bottle and consisted of weighing the cream and adding 9 cc. of water, plus 17.5 cc. of sulphuric acid. The third method consisted of adding 8–12 cc. of acid for a 9-gram and 14–17 cc. for an 18-gram charge, or acid was added until the mixture of acid and cream after mixing had a chocolate brown color. After thorough mixing not less than 5 cc. of hot soft water was added, whirled 5 minutes, soft hot water added to near top of neck and whirled 1 minute. Since 1925 A.O.A.C. (8) and since 1934 S.M.M.A. (210) have declared methods two and three as official. The specifications for the sulphuric acid are the same as those for milk. The temperature of the acid was not stated.

Hunziker *et al.* (117) and others (91, 113, 143) stated that the amount of acid varied with the fat content and sufficient acid of 1.82–1.83 specific gravity should be used to give a color similar to coffee after the cream had been added. Marquardt (143) stated that a 9-gram sample required 4–8 cc., and an 18-gram sample, 8–12 cc. of acid, and that after adding acid and mixing, water at  $82^{\circ}$  should be added to the base of the neck to prevent charring; Crowe and Davis (45) concurred in this method of retarding the action of the acid. Fuller (84) and others (135, 159) suggested diluting cream with 9 cc. of cold water and adding 9 cc. of acid, while Hunziker (116) and others (45, 122, 216) advised 9 cc. of acid for a 9-gram and 17.5 cc. for an 18-gram sample. Hunziker *et al.* (117) tested 96 samples of cream with acid and cream from  $5-43^{\circ}$ , and found no differences when the amount of acid was regulated by a color similar to that of coffee after the cream had been added. They concluded the best results could be obtained with acid and cream at  $21^{\circ}$ ; similar recommendations were made by others (143, 159).

### *Centrifuging Procedure*

*Milk.* The A.O.A.C. (8) in 1908, 1916 and 1920 stated that the centrifuge should be operated at a speed in accordance with the diameter of the wheel. In 1925 the A.O.A.C. (8) and in 1928 S.M.M.A. (210) indicated the

revolutions per minute for wheels of different diameters and the diameter was defined as the distance between the inside bottoms of opposing horizontal cups through the center of rotation. In 1922 the A.D.S.A. (3) specified an attached speed indicator, and the A.O.A.C. (8) in 1925 and S.M.M.A. (210) in 1928 adopted this recommendation.

Three whirling periods of 4, 2 and 1 minute, respectively, were stated by the A.O.A.C. (8) from 1908–1920 inclusive and by S.M.M.A. (210) in 1923, with the addition of “boiling” water between whirlings. In 1917 the A.D.S.A. (3) specified whirling periods of 5, 2 and 1 minute, respectively, with the addition of “soft” water at 60.5° or above, between whirlings, and the A.O.A.C. (8) in 1925 and S.M.M.A. (210) in 1928 adopted similar regulations except that “hot” water was stipulated for the second addition. For the first time the A.O.A.C. (8) in 1925 and S.M.M.A. (210) in 1928 stated that the centrifuge be heated electrically, or otherwise, to at least 55° during the whirling process.

The centrifuge described by Babcock (10) contained rigid trunnion cups that were inclined at an angle similar to the angle of inclination in De Laval’s (54, 55, 56) machine. The wheel that held the trunnion cups was surrounded by a stationary copper jacket with a cover. This jacket held hot water that could be further heated from beneath. Babcock (10) stated that the test bottles should not be heated to the boiling point of water in the centrifuge during the whirling process, but enough water should be poured in the jacket so that it would be heated to boiling when the whirling process is finished.

Babcock (10) stated that the first whirling should be 6 minutes, the second 1 to 2 minutes and the third for a short time with the addition of hot water between whirlings. He (13) later whirled the bottles for two periods of 5 and 1 minute, respectively, and Emery (63) followed this procedure. Weld (225) designated periods of 7, 3 and 2 minutes as most satisfactory. Farrington (74) and others (17, 21, 35, 90, 116, 143, 159) used centrifuging periods of 5, 2 and 1 minute, respectively, while Tolstrup and Mortensen (216) suggested whirling periods of 5, 2 and 2 minutes, and Fuller (84) recommended whirling periods of 5, 3 and 1 minute.

The temperature of the water added between whirlings has varied. Babcock (10, 14) implied the use of water near the boiling point, while others (17, 26, 74, 80, 91, 92, 175) specified the addition of hot water. Emery (63) recommended the use of soft water at 77° and preferably nearer 100° while Kerr (124) suggested a temperature of at least 82°. Liverance (135) and others (113, 116, 143, 212) suggested temperatures of 54° or above, while Jones and Wright (122) used water at 26.6°. The desirability of using water practically free from minerals to avoid the formation of insoluble salts was emphasized by most investigators.

Babcock (10) believed that heat from the chemical reaction was adequate

if the bottles were immediately whirled, but if they were allowed to cool to 37.6° or lower, he suggests heating to 93.3° before centrifuging and others (21, 35, 80, 135) followed this procedure. Bailey (17) reported that at room temperature milk will have the same reading from a heated or an unheated centrifuge, if whirled immediately after adding the acid and if water at 82.2° is added to the test bottles. Only small differences were obtained with either machine in a room at 10°. Fahl *et al.* (68) and Lucas (136) obtained higher results in heated than in unheated centrifuges; they suggested adding boiling water to the test bottles between whirlings if the room is cold; similar results were reported from the same station (7). Nelson (155) reported that estimations made in a room at 7.22° were 0.019 per cent lower than those made in a room at 28.9°, but he also stated with a temperature in the centrifuge of 37.8°, that 32 samples averaged 0.049 per cent higher than the Mojonnier estimations of the same milk and with the centrifuge at 54°, the samples averaged 0.07 per cent higher. Woll (237) and Farrington and Woll (77) found that a steam turbine centrifuge yielded results 0.1 to 0.3 per cent higher than the gravimetric method; these results were attributed to an abnormally high temperature in the centrifuge. Otis (162) reported that the unheated yielded results 0.2 per cent below the heated centrifuge. Woll (237) stated that manufacturers of centrifuges designed their machines to operate at high temperatures on the inside because they were of the opinion that such machines yielded the best results. Eckles (61) cautioned against the use of excessively hot centrifuges that heat the contents to near the boiling point, because impurities are more apt to be forced into the fat column. Burke (35) also suggested placing the test bottles from unheated centrifuges in water at 82° between whirlings for a few minutes.

Babcock (10) advocated whirling the test bottles at 600–800 r.p.m. and if the diameter of the wheel was less than 20 inches, the speed should be greater or the whirling time extended. Later (12) he proposed 700–1200 r.p.m., depending on the size of the wheel and that an 18-inch wheel should be run at 700–800 r.p.m. On this basis Farrington and Woll (80) calculated that a wheel operating at 800 r.p.m. would exert a force of 30.65 pounds, according to the formula,  $F = \frac{WV^2}{32.2r}$ , where  $F$  is centrifugal force in pounds,

$W$  is weight of test bottles and contents in pounds;  $V$  = velocity in feet per second; and  $r$  is radius of the wheel in feet. Using this formula they calculated the necessary speed for wheels of different diameters, that later appeared in a number of publications (21, 111, 113, 116, 159). Heinrich (93) reduced the speed of the centrifuge from 1500 to 800 r.p.m. with a subsequent reduction of 0.22 per cent fat and Bailey (17) reported similar results while the importance of adequate centrifugal force was further emphasized (27, 63, 143, 202).

*Cream.* The mechanical specifications outlined by the various official organizations for milk also apply to cream, with the exception of the whirling time. In method three for the addition of acid (see preceding section) only two centrifuging periods of 4 and 1 minute, respectively, were specified as contrasted to three periods of 5, 2 and 1 minute for one and two.

The whirling periods employed by the various investigators were similar to those for milk, with some exceptions. Marquardt (143) and others (21, 113, 122) recommended two whirling periods of 5 and 2 minutes, respectively. Bebee (30) suggested two periods of 5 and 4 minutes, and he found that longer periods were unnecessary.

The temperature of the water added between whirlings has been similar to that for milk, the chief distinction being that some investigators specified "hot" water, while others recommended water at 57° or above.

Bebee (30) placed a thermometer near the outer shell and maintained a temperature of 76.6° inside a steam centrifuge, thus he stated, the bottles were kept at 54.4–60°. Hunziker and co-workers (117) conducted tests in a cool and dry and in a steam-heated turbine centrifuge. There was no difference in the impurities in the fat columns and in the loss of volatile fatty acids between the two centrifuges. Webster (223) studied temperatures of 43.3, 48.8, 54.4 and 60° in the centrifuge. He weighed butterfat into test bottles and skim milk was added to a total weight of 18 grams, with a calculated butterfat content of 39.8 per cent. The average increase in reading was 0.5 per cent from 43.3–60°. He concluded that the hand-operated centrifuge gave more reliable results than the steam centrifuge because tests from the former were nearer the desired 48.8°. The tests were read directly from the centrifuge. Eckles (61) stated that the fat reading may be increased 1 per cent by raising the temperature from 43–82°, and similar results were reported by Farrington and Woll (78).

Hunziker and co-workers (117) conducted 547 tests in an 18-inch centrifuge to ascertain the effect of centrifugal force. They found that low speeds resulted in significantly lower tests and fat was observed in the liquid below the fat column. These results were corroborated by the recovery of larger amounts of fat from the acid hydrolysate when the speed was reduced. However, Siegmund and Craig (195) obtained lower results as the speed of their centrifuge was increased from 800–1000 r.p.m., and these lower results were attributed to the expulsion of occluded water and acid in the fat column. The diameter of the wheel was not given.

The first power-driven centrifuges according to Babcock (15) were of the steam turbine type, but during recent years the electrically powered and heated types are gradually displacing the steam machines. It is difficult to obtain information when the first electric centrifuges were marketed. The Chicago Department of Health (39) reported instructions for installing electric centrifuges in 1906. Kingsley (125) stated that a motor was at-

tached about 1900 and ten years later the heating element was included. Ziegler (239) stated from old catalogue files of the Cherry-Burrell Corporation, that the electric powered centrifuge was introduced sometime between the years 1900 and 1909, and the heating element appeared in the catalogue as standard equipment in 1923. Bingman (32) stated that the first electric centrifuges were marketed by The Jalco Motor Company in 1913 and the heating elements were included a short time later. Mojonnier (150) stated that his company sold electrically operated centrifuges soon after the year 1916, but this type of equipment was previously available from other companies.

#### *Temperature and Time of Holding Bottles in the Water Bath*

*Milk.* Babcock (10, 11) stated that reading the tests at 43.3–65.5° gave satisfactory results, but the higher temperatures were preferred. He stated that a difference of 22.2° made less than 0.1 per cent difference with milk containing 5 per cent fat. Farrington (74) and Emery (63) recommended tempering the tests to 60°, before reading and Emery (63) believed that one minute was sufficient. Bailey (17) read tests at 54.4°, but calculated this would cause an overreading of 0.032 per cent on milk testing 4.51 per cent, because he found the density of butterfat was 0.9 at 45°. Bailey (17) and others (35, 45, 116, 159) stated that the five minutes were sufficient. The A.D.S.A. (3) in 1917 specified not less than three minutes at 57–60°. Fuller (84) stated the readings could be made direct from a steam centrifuge. Harrison (90) estimated the fat content of 235 samples of milk and 2,068 samples of cream, using an electrically heated centrifuge, and reported no significant differences in the results when the tests were read direct from the centrifuge and from a water bath at 57° after five minutes. Caldwell and Herreid (37) employed water bath temperatures of 37.8°, 60° and 71° and obtained averaged tests of 4.33, 4.37 and 4.4 per cent, respectively, on the same samples of milk. Hays (91) believed it unnecessary to keep the bottles hot in the centrifuge.

*Cream.* The A.O.A.C. (8) in 1902 made no recommendations while in 1908 this organization cited Farrington and Woll (77) and Van Slyke (222) for procedures. The A.O.A.C. (8) for 1916 and 1920 specified the same procedure as for milk after weighing the cream, but also referred to Farrington and Woll (78) who stated that an error of one per cent (presumably bottle reading) may result due to the expansion of fat in an excessively hot turbine centrifuge with a tight cover, which they stated could be corrected by placing the test bottles in water at 60° for "some minutes." The A.D.S.A. (3) specifications for the time temperatures and exposure of the milk test bottles in the water bath, also apply to cream, with the exception that a few drops of glymol are added and the bottles are read at once with the aid of calipers.



Since 1925 the A.O.A.C. (8) has indicated that if glymol is used, a few drops should be allowed to flow down the inside of the neck just before reading each bottle; the specific gravity of the glymol should not exceed 0.85 at 20° and it may contain a soluble artificial color. The surface separating glymol and fat should be regarded as the upper limit of the fat column that must be free from visible foreign particles. The exposure of the bottle in the water bath is the same as for milk. S.M.M.A. (210) published these specifications in 1934.

Hunziker and co-workers (117) stated that the tests should be held in a water bath at 57.2–60° for five minutes because the bottles are calibrated for 0.9 as the specific gravity of butterfat at 57.2°. They obtained results 0.1 to 0.2 per cent too low, using water-bath temperature of 43.3–48.8°. Beach (28) obtained a reading of 0.42 per cent higher at 82° as compared to 54°. Eckles (61) stated that the exact temperature at which cream tests should be read had not been determined, but that it was near 49°.

#### *Method of Estimating the Fat Column*

*Milk.* The A.O.A.C. (8) in 1908 stated that the fat column should be read at 54–65.5° and in 1916 and 1920 to be read at 57–60°, and S.M.M.A. (210) contained similar specifications in 1923. No mention was made of the method to obtain these temperatures. The A.D.S.A. (3) in 1917 first specified a water bath, with thermometer and equipment to insure proper temperature control at 57.2–60°, and to leave the test bottles in the water bath for not less than three minutes. Since 1925 the A.O.A.C. (8) has stated that the test bottles, after the last centrifuging, should be immersed in the water bath at 55–60° to the level of the top of the fat column and left until the fat reached equilibrium and the lower surface had assumed final form. The bottle is wiped dry and the fat column measured with calipers or dividers from the lower surface to the highest point of the upper dimensions. The fat column must be translucent, golden yellow, or amber and free from visible particles. In 1928 S.M.M.A. (210) incorporated these specifications.

Babcock (10, 11) recommended that the fat column be measured in a perpendicular position with the line between the acid liquid and the column of fat in a horizontal position and the calibrations level with the eye, thus observing the highest and the lowest limits of the fat column. He (13) cautioned, "the reading should be taken at the line where the upper surface of the fat meets the side of the tube and not from the bottom of the dark line caused by the refraction of the curved surface." Babcock (10) stated that the fat column could be easily estimated to half divisions, which was to 0.1 per cent. Dahlberg (46) emphasized the difficulty of reading the meniscus and stated that the human eye cannot determine the extreme uppermost line of the meniscus. He is quoted, "one must assume the extreme upper end of the meniscus of the fat column to be that point at which

the fat seems to meet the wall of the glass rather than the high point to which the fat is drawn along the glass in a film by capillary attraction." Spillman (206) and others (21, 35, 45, 80, 84, 92, 113, 122, 135, 143, 159, 202, 212) recommended reading what they call the extremes of the fat column. White (226) employed better lighting conditions to show the outline of the meniscus more plainly and he thereby reduced the magnitude of the difference between the Mojonnier and the Babcock methods. Hortvet (110) devised an instrument for estimating fat columns, but it was not used extensively in commercial plants. Herreid (94) improved Hortvet's apparatus so that estimations could be made more accurately and under more standard conditions on both milk and cream. Theophilus and Barnett (213) reported the use of a device with a 60-watt blue bulb enclosed in a container with an opening from which the reading was made and a clear definition of the meniscus was obtained. Wilster and Robichaux (229) also reported a device to facilitate reading the fat columns. Mojonnier Brothers Company in Chicago, Illinois, list an apparatus to read the fat column, called the Wagner Junior Columnmeter, but it was not used extensively in commercial laboratories. This piece of equipment has mechanically adjusted calipers, but the points are blunt. Caldwell and Herreid (37) improved this device by replacing the dull points with sharp ones, illuminating the apparatus and adding a magnifying lens. Sanmann and Overmann (184) revealed significant personal differences in reading the Babcock test with the hand calipers. Nelson (156) stated that the probable error by the Babcock method was  $\pm 0.02$  per cent with possible variations of 0.1 per cent between different technicians, while Dahlberg (47) reported the experimental error on the same sample in different laboratories to be 0.2 per cent.

*Cream.* Winton and Ogden (235) in their earlier work read the fat column from the extreme top to the bottom. Webster (223) studied the dimensions of the meniscus and reported that readings from the top, middle and the bottom of the meniscus did agree with the gravimetric method. He suggested reading the extremes of the fat column, deducting four-fifths of the meniscus and adding 0.2 per cent to the result. He stated that the fat/water acid interface should be flat when the fat column was read. Liverance (135) advised including one-half of the meniscus. Eckles and Wayman (62) recognized the variability of estimating the large meniscus and recommended the use of amyl alcohol, colored red with fuchsin. Using this technique, the tests had to be read quickly because fat is soluble in the alcohol that in turn is miscible with water (208). The results showed close agreement with the gravimetric method. The chief disadvantage of this reagent is the toxic properties of amyl alcohol. Hunziker (114) advised reading to the bottom of the upper meniscus and adding one-third of the meniscus to the reading. Babcock and Farrington (16) and Smith (202)

recommended the use of fat saturated alcohol to eliminate the meniscus, while Hunziker *et al.* (117) after a thorough investigation recommended glymol, a mineral oil, colored red with alkanet root, that was favorably received by the industry. He also estimated cream tests to the bottom of the meniscus with the aid of a mirror to eliminate parallax and obtained close agreement with the gravimetric method. Doan *et al.* (57) estimated cream tests to the top and to the bottom of the upper meniscus, with glymol, with a fat-saturated alcohol and by including one-third of the meniscus. They found glymol to be the most reliable. Bebee (30) suggested the use of magnifying lens to aid in reading cream tests. Ross and McInerney (179) and others (21, 35, 45, 91, 143, 212, 216) advised the use of glymol.

#### *Modifications of the Babcock Method*

Attempts have been made to modify the Babcock test for the sake of expediency. Bartlett (26) submitted a procedure that consisted of using the usual charge of milk and 20 cc. of sulphuric acid of specific gravity 1.82-1.825. After the acid was added and the contents mixed with a rotary motion, the bottles were allowed to set for not less than 5 minutes, followed by a gentle shaking. Hot water was added to the uppermost mark and the bottles centrifuged at 1000 to 1200 r.p.m. for 5 minutes. A heated centrifuge was recommended. The data showed close agreement with the regular Babcock procedure. Hills (98) made 34 comparisons of the Bartlett modification with the Babcock procedure and likewise obtained close agreement but suggested its use by skilled testers.

Holm (106) was concerned with the constituents of milk that contribute to the charred material in the fat columns and stated that the carbohydrates cause a foamy char, the fats a brownish translucent char and the casein a flocculent brownish char. His method of preventing charred fat columns consisted of adding 2 cc. of a mixture of 80 parts glycerin and 20 parts water to the milk in the test bottle. When acid was added the glycerin formed a layer between the acid and fat to prevent charring before the bottle was rotated. No data were presented but he reported good results on several thousand samples.

Siegfeld (194) reported a modified method whereby the milk and sulphuric acid were mixed as usual, followed by 2 cc. of amyl alcohol. Next, sufficient sulphuric acid of specific gravity 1.5 and at 90-100° was added to fill the bottles to the uppermost part of the neck. The centrifuge was whirled for 3 minutes and the tests read. The results agreed closely with the Gerber and with the Adams gravimetric method.

James (120) suggested the addition of 3 cc. of a mixture of amyl alcohol and hydrochloric acid at the time the sulphuric acid was added and centrifuging at ordinary temperatures; thus making the procedure similar to the Beimling method.

*Comparisons of the Babcock and the Gravimetric Methods*

**Milk.** Babcock (10) compared his test with the Adams method using paper coils on 29 samples of fresh milk and the results showed that his test averaged 0.0193 per cent higher with 19 of the 29 comparisons agreeing within 0.10 per cent. Snyder (203) made 43 comparisons using asbestos as the absorbent in the Adams method and found that the Babcock method averaged 0.026 per cent lower; later (204) he reported that the Babcock method averaged 0.016 per cent higher. Results reported by Farrington (71), Dahlberg (50), Fisher and Walts (81) and James (120) show that the Babcock test exceeded the gravimetric ether extraction methods by 0.1 per cent or more, while the Iowa Experiment Station (6), Bailey (17), Dahlberg (48), Lucas (136), Phillips (172), Scratt-Fiecht (190), Shiver (196) and Shutt (198) reported that the Babcock test exceeded the gravimetric methods by less than 0.1 per cent. Close agreement was obtained by Mojonnier and Troy (151), Sebelien and Storen (193), Winton (231, 233) and Zehenter (238). Conversely, Patterson (168) reported that the gravimetric averaged 0.132 per cent higher, and Hortvet's (109) results were also 0.05 per cent above the Babcock method. On only 3 samples of milk, Hite (101) obtained higher results by the Adams method.

Bailey (17) obtained a reading of 0.179 per cent more than was contained in the neck of the bottle by including the extremes of the fat column. He calculated a net loss of 0.106 per cent in his experiments, and the difference of 0.073 per cent was above the gravimetric results. Hoyt (111) estimated the extremes of the fat column and by eliminating the upper meniscus with glymol he obtained close agreement with the Mojonnier method, while Phillips (172) using glymol obtained values 0.087 per cent below the Mojonnier and 0.146 per cent below the Babcock method when extremes of the fat column were included. Dahlberg (50) used glymol and obtained results 0.1 per cent too low as compared with a modified Røese-Gottlieb procedure. Jack and Abbott (118) tabulated the differences between results obtained by the Babcock and the various gravimetric procedures as reported by a number of investigators.

Smith (202) weighed purified butterfat into milk test bottles and added water to make a total of 18 grams. All but one of 16 estimations were too high when read to the top of the upper meniscus. He stated that the addition of a few drops of alcohol before reading gave results that compared favorably with the gravimetric method.

Since the earliest comparisons of results obtained by testing preserved and fresh samples of milk by the Babcock and by the ether extraction methods, there has been lack of agreement in the results obtained.

Campbell *et al.* (38), England and D'Ambrogie (65), Fahl *et al.* (68), Holland (104), Kochheiser (126), Meade and Leckie (146), Mecham (147), Monroe (152), Potts (173) and Sproule (209) all reported lower results

on the composite samples, while Babcock (15), Eaton (59), Farrington (73), Ronneberg (178), Neumann (158), Sanmann and Overmann (182), Windisch (230) and Zehenter (238) obtained results that were only slightly lower or agreed with the daily samples. Sanmann and Overmann (183) submitted data to show that composite samples stored at 10°, or below agreed closely with the daily tests. They (183) further showed that samples stored in the receiving room when the temperature varied from 30–38.8° tested lower than those held at 7.2–10°, the differences increased as the storage period was prolonged. Similar results were reported by Campbell *et al.* (38) and others (104, 209). Tracy and Tuckey (218) reported that daily tests on fresh samples agreed closely with their experimental composites, but the routine plant composites tested lower. Holland (104) reported declines of 0.08–0.12 per cent fat in composite samples that he attributed to destabilization of the fat emulsion and which could be avoided by the addition of 0.5 grams of saponin to 250 cc. of milk. Wilster (229) on the other hand, reported no beneficial results from the use of saponin.

With the rise in popularity of homogenized milk, it was necessary to estimate the fat content for commercial purposes. It was observed by Hollingsworth (105) and others (9, 108, 112, 180, 217) that the fat content of homogenized milk was lower than that of the original raw milk by the Babcock method. Trout (219) reported curdy or charred material below the fat column with the regular Babcock procedure, and he found that the specific gravity of this material was higher than the milk fat and was of a tenacious nature. Tracy (217) reported that this condition could be alleviated by adjusting the milk and acid to 21.1° before mixing and by adding the acid in small equal portions to a total of 16 cc. Ruehe (180) stated that if this method is used and the acid is added in five equal portions and the contents shaken thoroughly after each addition, there should be no difference in the results from homogenized and from normal milk. Hood and White (108) obtained clear fat columns with both the acid and the milk at 5–10° and shaking the test bottles thoroughly after the first and after the second whirlings. They reported that milk homogenized at 3500 pounds tested 0.08 per cent lower than the normal milk. Roadhouse and Henderson (177) published essentially the procedure suggested by Hood and White (108) except that the temperature of acid and milk was not stated. The explanation for the lower results from homogenized milk has been ascribed by Hollingsworth (105) to such a fine subdivision of the fat globules that the smaller ones are not separated by the centripetal force. On the contrary, Doan and Swope (58) stated that homogenization had but little influence on the Babcock test and Halloran and Trout (89) reported similar results.

*Cream.* Babcock (10) compared his method with the Adams gravimetric method on four samples of cream and obtained close agreement, presumably

the whole milk test bottle was used. Hortvet (109) reported that the Babcock averaged 0.4 per cent higher than the Röse-Gottlieb. Ross and McInerney (179) reported results on 58 samples of fresh cream by the Babcock method, using glymol to eliminate the meniscus and a method they called chemical, presumably an extraction method. Both methods checked well, with only eight of the tests varying more than 0.50 per cent. Schrott-Fiechtl (191) reported that the cream test bottle yielded results that compared favorably with the gravimetric method. Close agreement was reported by Fisher and Walts (81) for the Babcock and Mojonniér methods.

Dahlberg (50) estimated the fat content of 34 samples of cream by the Babcock and the Röse-Gottlieb methods and found that the Babcock averaged 0.13 per cent higher, while Mojonniér and Troy (151) reported that the Babcock method averaged 0.33 per cent higher on seven samples of high fat content cream. Doan *et al.* (57) obtained a difference of 0.28 per cent higher with the Babcock method.

Dahlberg *et al.* (48) reported results from three laboratories on 33 samples of fresh cream that showed the average variation of the Babcock from the Röse-Gottlieb was  $-0.32$ ,  $+0.20$ , and  $+0.34$  per cent. Siegmund and Craig (195) obtained higher results by the Babcock method; they also reported that several additional ether extractions in the gravimetric method did not make any appreciable difference.

Hunziker *et al.* (117) compared daily and preserved composite samples of cream and both agreed when the preserved samples were handled correctly. The composite yielded results that were too high when they were not tightly stoppered, and also when tightly closed but exposed to high temperatures. Lee and Hepburn (129) reported greater variation between composites and individual samples than between two sets of composites, and that there was a tendency for composites to test slightly higher than daily samples in the summer and lower in the winter. Combs *et al.* (41) reported that when composites were prepared in aliquot the producers could expect accurate tests in 60.8 per cent of the deliveries, but the dipper method reduced this accuracy to 45 per cent of the deliveries.

Martin *et al.* (145) in a comprehensive statistical study, from 1599 estimations made on samples obtained from ten gallons of sweet cream and the same cream allowed to sour, found that 97.5 per cent of the readings were approximately within 0.5 per cent of the mean.

#### DISCUSSION

The early literature revealed much research on volumetric methods of estimating fat in milk before Babcock gave his test to Dairy Science. Babcock's test was a simplification over early methods so that it had immediate practical application. His essential contribution was the use of the one reagent, sulphuric acid, and the application of specified whirling periods with the addition of hot water between whirlings.

The Babcock test originally was standardized for accuracy by comparison with the ether extraction gravimetric method. Some of the comparisons reported in the literature between the Babcock and the gravimetric methods agreed closely, while most of the Babcock results are above and a few below the gravimetric method. The results may be complicated by variable technique in the conduct of the official methods. For example in the laboratory of the Vermont Station it has been shown that weighing four 10-cc. pipettes full of the same milk on the weighing stand in the Mojonnier method, yields lower results for the milk from pipettes numbers 2, 3 and 4: Creaming of the milk with adsorption of the cream to the pipettes during the process of weighing is responsible for these lower results. Pipette number 1 does not remain long enough on the weighing stand for the cream to rise sufficiently so that the results check closely with those obtained by weighing milk directly into the extraction flask. It should be stated that the temperature of the ethers and of the milk may also be a factor of some importance.

The fact that the ether extraction methods also include a portion of the phosphatides in milk, further complicates the comparisons between the Babcock and the official method. Theoretically, the Babcock method should give results that are too high because it was designed to agree with the official procedure. It should also be recalled that the earlier comparisons between the gravimetric and Babcock methods were made when the test bottle was calibrated in 0.2 per cent divisions, thus allowing considerable latitude in interpreting the results.

It is questionable if all the gravimetric methods agree on milk and cream. Theoretically, the gravity separation of the fat-ether solution in the Röse-Gottlieb method should not be as exhaustive as the centrifugal separation in the Mojonnier procedure.<sup>5</sup> It has been observed in the laboratory of the Vermont Station that the Mojonnier vacuum drying oven, operating at 57°, has a small amount of fat in the uppermost surface after a number of determinations have been made; however, the degree of volatilization of fat from each determination must be small so that the error involved may not be great.

The temperature of sampling milk affects the reliability of the results obtained by the Babcock method. Fresh whole milk can be sampled at lower temperatures than preserved milk, because the effect of storage and the action of the preservative changes the physical-chemical nature of the fat emulsion. Therefore, it is necessary to apply heat in order to render the emulsion of fat homogeneous in preserved composites so that a representative sample can be obtained. The composites must be heated to at least 32.2°, and some state regulations specify up to 43°. Composites are also susceptible to handling, consequently, they must be carefully prepared to

<sup>5</sup> Mechanized modification of the Röse-Gottlieb method.

avoid separation of the fat. If they are prepared at 15.6–21.1°, partial churning is apt to occur and the partially churned fat will adhere to the inside of the pipette and the results will be too low.

Another variable factor in comparing the Babcock and the gravimetric methods was the amount of milk used in the Babcock test. Some investigators (17, 50, 111) used pipettes that were standardized to deliver 17.6 cc. of water in 5–8 seconds and this was generally assumed to be the intent of calibration prior to the year 1917. One investigator (172) weighed 18 grams of milk into the test bottles. Since 1925 the A.O.A.C. (8) has specified that the pipette shall contain 17.6 cc. of water at 20°. The method of interpreting the capacity of the pipette has caused some confusion. Babcock (10, 11) considered that the pipette was full when the milk touched the mark on the draw tube, while it is a universal practice for technicians to consider a pipette full when the lowest part of the meniscus is level with the mark.

The centrifuge has undergone considerable change since the inception of the Babcock test. The transition has been from the unheated hand operated to the electrically powered and heated centrifuges. The centrifuge described by De Laval (54, 55, 56) was operated at 50°, while the machine described by Babcock (10) had provision for applying heat. However, a large number of centrifuges were used that could not be heated and finally, the commercial impetus for the use of the heated machines came from the manufacturers who believed that they gave the best results. Many of the early comparisons between the gravimetric and Babcock methods, were made with the unheated centrifuge, and in some instances the whirling mechanism was not inclosed, with trunnion cups and bottles revolving in the open air; thus the contents of the bottles would cool rapidly and subsequent work has indicated that under these conditions the Babcock method yields lower results. Fahl, Lucas and Baten (68, 136) obtained lower results with the unheated as compared to the heated centrifuge, which was verified in the laboratory of the Vermont Station. Thus some of the work reported is complicated by the type of centrifuge used, whether heated or unheated. It is known that some steam-powered centrifuges may operate warmer than others due to the leakage of steam into the chamber. On the other hand, a steam operated centrifuge, open on the top center and not leaking steam, will operate cooler and the results may be lower than those obtained with a heated machine. It is also possible for steam centrifuges to operate too hot. For example, excessive steam leakage into the whirling chamber will heat the test bottles and contents abnormally high and when they are placed in a water bath at 57–60° the contents will recede to a point where the bottom of the fat column is below the lower graduation, thus legal reading is impossible. This condition may be further aggravated by some state regulations that specify the addition of water at 82° or above, between



whirlings, or even require that the centrifuges be operated at higher temperatures. Because of the excessive recession of the fat column, the dimension of the meniscus may be greater and some fat may stick to the glass and thus not be estimated. To again bring the fat column within the graduated scale it is necessary to add water and whirl for about one minute.

The speed of the centrifuge is of importance. If the steam pressure decreases, the speed of the centrifuge is likewise decreased. A similar condition occurs in the electric powered centrifuge when the voltage drops. It was observed in one plant that the speed of the centrifuge decreased when all of the electrically driven equipment was in operation, indicating inadequate power requirements; this would undoubtedly affect the results obtained by the Babcock test. The A.D.S.A (3) in 1922 and the A.O.A.C. (8) in 1925 specified an attached speed indicator, but only one reference (229) reported a centrifuge equipped with a permanently attached tachometer. Some technicians have been observed to judge the desired speed of the centrifuge by the pressure gauge reading, but this is an unreliable procedure.

The water bath was originally intended to standardize the method of estimating the fat columns of a large number of bottles when the unheated centrifuges were used and when laboratories were colder than they are at the present time. The revolving of the centrifuge wheel is a cooling process and the degree of cooling is dependent upon the temperature of the room and whether or not the centrifuge is open or closed in the center. It has been observed that even a modern centrifuge, electrically powered and heated, will decrease in temperature during the first five minute whirling period. It may be possible to eliminate the water bath when readings are made from centrifuges heated to 55–60° in laboratories at 21.1° or above.

The test bottles should be more finely calibrated to reduce the latitude in interpreting the results. A consistent difference of 0.1 per cent in results between two technicians is too much variation for the sake of plant efficiency in accounting for milk fat, and this is within the legal tolerance at the present time. Furthermore, the reading of the Babcock test should be placed on a more scientific basis. There is difference of opinion about the magnitude of the meniscus when estimations are made with the hand calipers in commercial plants. This variable factor can be eliminated by the use of glymol, but the amount of milk used in test will have to be increased so that the results will agree with the official method. The use of the improved Hortvet apparatus (94) has resulted in close agreement between the Babcock and Mojonnier methods (95) with the latter averaging about 0.02 per cent lower. The dimensions of the meniscus can be accurately measured with this device.

The Babcock test is somewhat empirical and some refinements are necessary in order that it may continue to be acceptable to the Dairy Industry and maintain the confidence of all concerned. In this connec-

tion it should be stated that the Gerber test is gaining popularity because of alleged simplicity and speed of obtaining results. The states of New York and New Jersey have legalized this method. It is possible that the Babcock test may be modified so as to shorten the procedure, without sacrificing accuracy.

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\* Title has been changed, slightly.

## American Dairy Science Association Announcement

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March 9, 1942

To the Members of the  
American Dairy Science Association:

I am happy to report to you that our committees have nearly completed their plans for the meeting of the Association here at Michigan State College June 22-26. Our dairy staff as well as the numerous other members of the Association located in Michigan extend you the invitation to be with us.

Those of us here are fully aware that unusual conditions confront our members this year as they contemplate any trip. We are even reconciled to a possibility that some may not be able to come to East Lansing. Nevertheless, our committees are proceeding in a belief that an especial obligation devolves upon us this year, more than in normal times, to provide a profitable and memorable meeting.

The May number of the JOURNAL OF DAIRY SCIENCE will furnish you the detailed program for the meeting. You will note from the program that first attention centers on those questions of especial import to dairy workers today. The role our industry plays in our national emergency makes it imperative that we dairy workers keep apace. Some persons express a conviction that never before in our experience has there been a more urgent need for dairy workers to meet for conferences on technical topics and for discussion and dissemination of research results pertaining to our industry. And all of us are involved whether we be engaged in research, in instructional work either resident or extension, in plant operation or engaged in regulatory activities.

Shortly you will receive from our publicity committee some useful material pertaining to the meeting and Michigan. This will be of interest to your families also. In due time our Registration Committee will communicate with you. Your cooperation is solicited in complying with the requests this committee will make of you.

Sincerely,

EARL WEAVER,

*Head Dairy Department,  
Michigan State College*





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## THE RELATIONSHIP OF ERRORS IN THE BABCOCK TEST TO LOSSES IN CREAM PLANTS

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An economic factor of great importance in the operation of cream plants is the loss of butterfat. The significance of certain errors in the Babcock test in contributing to such losses has apparently not been recognized.

Numerous comparisons of fat tests by the Babcock and Roese-Gottlieb or Mojonnier method have been published for both milk and cream. Tables 1 and 2 show the averages of all of these comparisons that have come to the attention of the authors, including some previously unpublished data obtained in these laboratories.

TABLE 1

*Comparisons between Babcock and Mojonnier tests on fluid milk*

Reference	Number of samples	Babcock test	Mojonnier test	Difference	Difference expressed as per cent of Babcock test
(1)	1	4.409	4.365	- 0.044	
(2)	190	4.497	4.438	- 0.059	
(3)	60	4.000	3.840	- 0.160	
(4)	32	4.770	4.670	- 0.100	
(5)	30	3.601	3.611	+ 0.010	
(6)	513	3.757	3.675	- 0.082	
(7)	16	4.118	4.061	- 0.057	
(8)	3	3.533	3.444	- 0.089	
(9)	14	3.783	3.809	+ 0.026	
(10)	36	4.204	4.088	- 0.116	
(11)	14	4.230	4.210	- 0.020	
(12)	50	3.881	3.822	- 0.059	
(13)	21	4.275	4.242	- 0.033	
(14)	900	4.548	4.472	- 0.076	
Average from literature ...	1880	4.257	4.181	- 0.076	
Hileman <i>et al.</i> ...	149	3.484	3.411	- 0.073	
Grand average	2029	4.200	4.124	- 0.076	1.80

For details of the technique of performing the Babcock and Mojonnier tests in the work cited from the literature, the original articles should be

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consulted. It might be well to note that the comparisons made by Dahlberg, Holm and Troy (5) were complicated by the facts that the samples were chemically preserved and were transported, in part at least, by mail. So far as is known, all other samples were not chemically preserved. Wilster and Robichaux (14) have reported some comparisons on preserved samples

TABLE 2  
*Comparisons between Babcock and Mojonnier tests on cream*

Reference	Number of samples	Babcock test	Mojonnier test	Difference	Difference expressed as per cent of Babcock test
(4)	34	36.931	36.800	- 0.131	
(5)	33	32.360	32.290	- 0.070	
(15)	10	26.475	26.193	- 0.282	
(7)	4	32.047	32.087	+ 0.040	
(9)	14	18.621	18.182	- 0.439	
(16)	23	39.774	39.067	- 0.707	
(17)	24	33.968	33.966	- 0.002	
Average from literature . . . .	142	33.149	32.925	- 0.224	
Hileman <i>et al.</i> . . .	186	38.160	37.668	- 0.492	
Grand average.	328	35.991	35.615	- 0.376	1.04

also, but only their 900 comparisons on fresh samples have been included in table 1.

For the comparisons made in these laboratories, here published for the first time, all Babcock testing of milk was done in exact accordance with the procedure specified by the New York State Department of Agriculture and Markets (18). All glassware was rechecked at the Experiment Station at Geneva and bore the "SB" designation. Sulphuric acid of density 1.825 to 1.828 at 60° F. was used. All tests were in duplicate, and no results were used unless each of the two duplicates had a perfect fat column and the two agreed within 0.1 per cent. All tests were read after tempering at least 5 minutes in a water bath at 135-140° F. Water added to the bottles to bring the fat column up into the neck was at 165° F.  $\pm$  3°, which is now recognized as correct for heated centrifuges in New York State, although it was not so recognized during all of the several years during which the data were being accumulated (19).

Babcock tests on cream were made in these laboratories also in exact accordance with the procedure outlined by the New York State Department of Agriculture and Markets, with two exceptions. Water used to bring the fat columns up into the necks of the bottles was at a temperature of about 165° F. (See the last sentence of the preceding paragraph.) The bottles used were especially made, nine inches long instead of six inches long, and with the necks graduated to read up to 60 per cent fat. Because of the longer graduated necks, tests could easily be read to 0.25 per cent. These

bottles were all rechecked by the New York State Agricultural Experiment Station at Geneva, New York, and any showing an error greater than 0.25 per cent were rejected. This is only half the error that is allowed for state-branding of ordinary Babcock cream test bottles. Numerous comparisons with state-branded 50 per cent six inch cream test bottles showed that the longer bottles gave identical results, within the limits of accuracy of the Babcock test. All tests were in duplicate, and only perfect fat columns were read. Only results where duplicates agreed within 0.50 per cent were used. All tests were read after tempering for 5 minutes in a water bath at 135–140° F. "Red reader" was used to flatten the upper meniscus.

Mojonnier tests were run in exact accordance with the procedure outlined in "The Technical Control of Dairy Products" by Mojonnier and Troy (11), except for certain special precautions. Ethers were all redistilled. Milk samples were either weighed directly into the fat-extraction flasks or pipetted from high-grade carefully re-calibrated 10 ml. chemical pipettes, with fine tips to insure uniform delivery. The latter method, using a cold sample to avoid possibility of churning, has proved to be the most satisfactory for measuring fluid milk samples for Mojonnier tests. Warming samples is apt to cause enough churning to be significant in Mojonnier tests. Weighing samples in pipettes often allows formation of gravity cream in the pipette which may stick to the walls of the pipette and cause appreciable errors. It is obvious that the calibration of the pipette should be with milk at the same temperature as that used when the samples for analysis are transferred to the flasks.

Cream samples for Mojonnier tests were weighed in pipettes, the samples being at room temperature. A third extraction, using 15 ml. of each ether, was used with cream. All Mojonnier tests were in duplicate, and only those tests with duplicates agreeing within 0.03 per cent for milk, and within 0.25 per cent for cream, were used. Most of the duplicates were much closer than these extreme limits.

The "difference expressed as per cent of the Babcock test" is 1.80 for milk. This means that, for every 100 pounds of fat in milk purchased, the purchaser pays for 101.80 pounds. If this milk is made into cream and sold, the manufacturer gets paid for 101.04 pounds of fat. The difference, or 0.76 pounds, represents a loss of 0.76 per cent which cannot be avoided so long as present methods of Babcock testing are used.

It seemed desirable to determine if the richness of the milk skimmed and of the cream produced has any bearing on the magnitude of this loss. In order to do this, it was decided to analyze the data of Fahle, Lucas and Baten (6) on milk, and of the authors on cream. These particular sets of data were chosen in order to have a large number of comparisons covering a wide range of fat content. It would be better to have the data for both milk and cream from a single laboratory, but there appears to be no work available

TABLE 3  
*Variation of the difference between Babcock and Mojonnier tests on milk with variation in fat test. From work of Fahle, Lucas and Batten (6)*

Range of Babcock tests	Number of samples	Average Babcock test	Average Mojonnier test	Difference	Standard deviation of the difference	Probable error of determining the average difference	Difference expressed as per cent of Babcock test
3.00-3.49	143	3.341	3.282	-0.059	0.079	+0.0044	1.76
3.50-3.99	254	3.705	3.624	-0.081	0.091	+0.0038	2.18
4.00-4.49	79	4.218	4.120	-0.098	0.092	+0.0070	2.32
4.50-4.99	32	4.666	4.534	-0.132	0.111	+0.0132	2.82
5.00 and up	5	5.126	4.933	-0.193	0.047	+0.0142	3.76
Average	513	3.757	3.675	-0.082	0.092	+0.0021	2.18

TABLE 4  
*Variation of the difference between Babcock and Mojonnier tests on cream with variation in fat content*

Cream standardized to	Number of samples	Average Babcock test	Average Mojonnier test	Difference	Standard deviation of the difference	Probable error of determining the average difference	Difference expressed as per cent of Babcock test
20	45	20.317	20.159	-0.158	0.298	+0.030	0.77
40	88	40.171	39.644	-0.527	0.280	+0.020	1.31
50	53	49.972	49.255	-0.717	0.324	+0.030	1.43
Average	186	38.160	37.668	-0.492			1.28

which meets the above requirements. The authors have data, shown in tables 1 and 2, for both milk and cream, but the range of fat content for the milk samples is very narrow.

Tables 3 and 4 show the results of analyzing these data, as discussed above.

From an examination of tables 3 and 4, it is obvious that the difference between the Babcock and Mojonnier tests increases with the fat content for both milk and cream, and that the difference expressed as per cent of the Babcock test is smaller for cream than for milk. This means that the skimming loss due to the error in the Babcock test will increase with increasing fat content of the milk skimmed and will decrease with increasing fat content of the cream produced. Table 5 shows this more in detail.

TABLE 5

*Variation in per cent skimming loss due to errors in the Babcock test with variation in fat content of milk skimmed and cream produced*

Fat test of cream	Fat test of milk					
	3.34	3.70	4.21	4.66	5.12	Average 3.75
20.317	0.99	1.41	1.55	2.05	2.99	
40.171	0.45	0.87	1.01	1.51	2.45	
49.972	0.33	0.75	0.89	1.39	2.33	
Average 38.160						0.90

Another source of loss in the manufacture of cream, which is often overlooked because of an error in the Babcock test, is fat in the skim milk. Table 6 shows a comparison of Babcock and Mojonnier tests on skim milk. In the case of milk and cream, the Mojonnier test is slightly lower than the Babcock. In the case of skim milk, however, the Mojonnier test is about seven times as high as the Babcock. For efficiently operated separators, the Babcock method gives a test of about 0.01 per cent on the skim milk. This is so low that much butterfat accounting ignores it entirely. However,

TABLE 6

*Comparisons between Babcock and Mojonnier tests on skim milk*

Reference	Number of samples	Babcock test	Mojonnier test	Difference	Difference expressed as per cent of Babcock test
(4)	15	0.0140	0.0870	+ 0.0730	
Hileman et al. ....	60	0.0102	0.0691	+ 0.0589	
Average .....	75	0.0109	0.0726	+ 0.0617	566.05

the true fat content by the Mojonnier test is about 0.07 per cent. If milk testing 3.5 per cent fat is skimmed, this means that 2 per cent of the fat will not be recovered in the cream. Failure to recognize its presence in the skim milk means that there will be an unexplained loss of 2 per cent of the fat purchased.

An illustration of how these factors affect losses in an actual cream plant is given by an experiment. During a fifteen-day period, the milk and cream in a certain cream plant were carefully weighed, and both were tested by both Babcock and Mojonnier methods. The dry skim milk produced was also tested by the Mojonnier method. Table 7 gives a comparison of losses by the two methods of testing.

TABLE 7

*Losses in a cream plant on the basis of Babcock and Mojonnier tests, for a 15-day period*

	Mojonnier test	Babcock test
Pounds milk skimmed	826,425	826,425
Average test of milk	3.38395	3.46042
Pounds fat in milk	27,965	28,597
Pounds cream made	68,725	68,725
Average test of cream	39.71335	40.21971
Pounds fat in cream	27,293	27,641
Per cent of the fat in the milk recovered in the cream	97.60	96.65
Pounds dry skim milk made	67,050	Neglected
Average test of dry skim milk	0.74407	
Pounds fat in dry skim milk	498	
Per cent of the fat in the milk recovered in dry skim milk	1.78	
Pounds fat lost	174	956
Per cent fat lost	0.62	3.35

The Babcock test, with the fat in the skim milk neglected, shows 782.9 pounds more loss than does the Mojonnier. Of this amount, 284.0 pounds are due to the error in the Babcock test for milk and cream, equivalent to 0.99 per cent of the fat in the milk. The fat in the skim milk, 498.9 pounds, is equivalent to 1.74 per cent of the fat in the whole milk. The fat actually lost was only 173.9 pounds, equivalent to 0.62 per cent of the fat in the whole milk.

#### SUMMARY

The Babcock test gives a result that is too high in the case of both milk and cream. Because the error is proportionately greater in milk than in cream, a loss results. This loss increases with increasing fat content of the milk skimmed, and decreases with increasing fat content of the cream produced. It may vary from about 0.35 per cent where cream testing 50 per

cent fat is made from milk testing 3.35 per cent fat, up to about 3 per cent where cream testing 20 per cent fat is made from milk testing 5 per cent fat.

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# EFFECT OF INHALED SUBSTANCES ON MILK FLAVORS<sup>1</sup>

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Most, if not all, of the commercially-important flavors of milk are exogenous; at least, there is no evidence that the synthesizing mechanism of the udder produces any of these flavors. This concept requires that the flavoring substance is present in the blood and the "membranes" of the mammary gland are permeable to it. There are three possible avenues through which flavoring substances may gain entrance to the blood: (a) through the walls of the alimentary tract from ingested material; (b) through the lungs from inhaled substances; and (c) through the skin from contacted substances. The further possibility also exists that flavors or odors from ingested material may gain entrance to the blood by passing progressively from the point of rumination to the lungs and then into the blood.

This report will deal with some preliminary experiments in a study of the effect of certain inhaled substances upon the flavor of milk. While there is a considerable amount of literature (2) dealing with the effect of different ingested substances upon milk flavors, there is a scarcity of information upon the effect of inhaled substances. Aside from the general knowledge that milk from animals recently anesthetized with ether or chloroform contains these anesthetics and the report by Babcock (1) that inhaled odors of wild garlic tops could be detected in the milk, the authors are unaware of any reports in the literature dealing with the specific effect of inhaled substances upon milk flavors. It is rather common knowledge, however, that inhaled gases such as methane, nitrous oxide, hydrogen cyanide, chlorine, bromine, etc., and vapors from many compounds pass through the lungs into the blood. Because of these facts and the fact that the mammary gland behaves as a permeable membrane to many substances in the blood, it was thought advisable to begin an investigation of the effect of certain inhaled substances upon milk flavors. The substances investigated and reported herein are: turpentine, paradichlorobenzene, benzaldehyde, camphor, vanillin, synthetic orchid, onions, garlic, manure, corn silage, alfalfa silage and scrapings from Roquefort cheese.

## PLAN OF THE EXPERIMENT

The essential features of the experimental plans upon which comment is needed are: (a) the special stalls, (b) the method of administering the

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<sup>2</sup> Now at Cornell University.

substance to be studied, (c) the milking and sampling, and (d) the judging of the milks.

*The Stalls.* In order to confine the odors to the head regions of the animal and prevent, insofar as possible, absorption of the odors on the posterior of the body, which might contaminate the milk when drawn, a special stall was constructed. The manger was completely enclosed in a "tent" approximately  $4\frac{1}{2} \times 2\frac{1}{2} \times 3\frac{1}{2}$  feet in dimensions. By first putting in wood partitions between the mangers, the "tent" was completed by covering with a heavy duck canvas, cut so as to fit snugly around the animal's neck at the stanchion. A heavy wire screen was fitted over the lower part of the manger in such a manner that at least two inches intervened between the solid materials to be tested and the cow's mouth to insure that none of the substances could be taken by mouth. In order to prevent contamination of the milking barn with odors, the stalls were constructed in a barn separate from where the milking took place.

*Method of Administration.* In all trials the cows were placed in the tent and inhaled the odors or vapors for two hours immediately before milking. They were then removed to the milking barn. For comparative purposes, turpentine, benzaldehyde, camphor, vanillin and onions were administered orally two hours before milking. The solid substances, paradichlorobenzene, camphor, onions, garlic, manure, corn and alfalfa silage and scrapings from Roquefort cheese, were placed in the manger and covered with the heavy screen immediately before the animal was placed in the stall. The fluid substances, turpentine, benzaldehyde, spirits of camphor, and vanillin solutions (in alcohol) were placed in the manger on cotton for one series of experiments and for another were atomized into the tent. Synthetic orchid was tried only by atomizing. The onions and garlic were crushed before being placed in the manger and the silages were stirred periodically to free a maximum of the volatile substances into the tent.

The mangers were thoroughly washed and aired after each experiment. In the case of paradichlorobenzene, it was not possible to rid the manger completely of this compound and no further experiments were conducted in the stall where it was used.

*Taking the Milk Samples.* The cows were milked by machine and a quart sample was taken from the completed milking for the judging. The sampling was done in a milk room which was free from contaminating odors. One or more samples from the normal herd cows were taken simultaneously in a similar manner for comparison purposes.

*Judging of the Milks.* From three to five people,<sup>a</sup> experienced in judging, were used in rating the milks from all trials. The judges had no

<sup>a</sup> The authors are indebted to members of the Dairy Division staff who contributed their time and ability in judging the milks reported herein.

knowledge of the substance investigated, all samples being identified by number, the identity of which was known only to the authors. The mixed herd samples were likewise unknown to the judges. The judges, working independently, not only decided as to whether or not the milk had abnormal flavors but they also commented upon the character of the flavor.

#### RESULTS AND DISCUSSION

It was noted that the head and that portion of the neck which was inside the "tent" had absorbed the characteristic odor for each substance tested whether or not such could be detected in the milk. The posterior part of the animal's body was free from any absorbed odors insofar as could be ascertained.

A summary of the effects on milk flavor of the substance studied by the various methods of administration are given in table 1. A discussion of the results obtained for each of the substances tested follows:

*Turpentine.* Whether turpentine was placed on cotton in the bottom of the manger, atomized into the tent or given orally (30 ml. quantity), the milk was pronounced abnormal by all judges in all cases. In the trials where the turpentine was placed in the bottom of the manger, the flavors were less pronounced than where administration was by atomizing or by mouth. In the former, the judges declared that the milks were "aromatic" or "medicinal," while for the trials in which the turpentine was administered by atomizing the judges unhesitatingly pronounced the flavor as that of turpentine or closely allied substances, such as varnish or paint. In the one trial where the turpentine was administered orally, the turpentine flavor of the milk was very distinct as indicated by the fact that all three judges described the flavors as "turpentine."

*Paradichlorbenzene.* Inhalation of the odors from paradichlorbenzene had such a pronounced effect upon the flavor of the milk that only two trials were necessary to establish the fact that this substance passes through the lungs to find its way ultimately into the milk. The flavor of the milk is unmistakably that of the chemical.

*Benzaldehyde.* In one trial, involving two cows, benzaldehyde was placed upon cotton in the manger. One judge declared that the milk had a sweetish taste while three judges proclaimed the milk normal. When benzaldehyde was atomized into the "tent" all three of the judges distinguished these milks from the normal or control milks and described them as "aromatic" or, in one instance, as "off flavor." When one ml. of benzaldehyde was administered orally, the judges described the milk as being "sweetish" and "aromatic."

*Camphor.* Pulverized camphor, placed in the manger, and atomized spirits of camphor produced a decided "medicinal" or "aromatic" flavor, while one judge detected an actual camphor flavor in one case. When

TABLE 1  
Summary of the effect of various inhaled or ingested substances upon the flavor of milk

Substance	Method of application	No. of cows	Judges' report		Comments of judges
			Normal No.	Abnormal No.	
Turpentine	Bottom of manger	2	0	4	Aromatic, medicinal
"	Atomized	2	0	5	Turpentine by 3 and aromatic
"	29 ml. orally	1	0	4	Paint, varnish, turpentine
Paradichlorobenzene	In manger	2	0	3	Turpentine by all 3
"	In manger	1	0	4	Aromatic, moth balls, musty
Benzaldehyde	In manger	2	0	3	Aromatic, moth balls, musty
"	Atomized	2	3	1	Sweetish
"	2 ml. orally	2	0	3	Aromatic, off flavor
"	4 ml. orally	2	0	3	Sweetish, vanilla-like
Camphor	In manger	2	0	3	Sweetish, aromatic
Spirits of camphor	In manger	2	0	4	Medicinal, off flavor
"	Atomized	2	0	4	Medicinal, aromatic
"	58 ml. orally	2	0	3	Medicinal, camphor
Vanillin	Atomized	2	0	5	Camphor by 4, medicinal
"	58 ml. orally	1	0	3	Vanilla by all
"	29 ml. orally	1	3	0	Slightly vanilla
Synthetic orehid	Atomized	1	3	0	
Crushed onions	In manger	2	0	4	Sl. objectionable flavor
"	In manger	1	1	3	Feed, sweetish, sl. off
Onions	28.3 gm. orally	1		4	Onions, undesirable
"	453.5 gm. orally	1		3	Strong onion
Crushed garlic	In manger	2	3	1	Sl. feed flavor
Manure	In manger	2	1	3	Cowry, objectionable
"	In manger	2	1	2	Cowry, unclean
Corn silage	In manger	2	1	3	Feed flavor
Alfalfa silage	In manger	2	1	3	Feed flavor, unclean
Alfalfa strong odor	In manger	2	0	3	Feed flavor by all
Scrappings from Roquefort cheese	In manger	2	3	0	

approximately 58 ml. of spirits of camphor were administered orally to two cows, four judges noted a camphor flavor in the milk and the fifth judge termed the flavor "medicinal."

*Vanillin.* Atomized vanillin, dissolved in alcohol, caused the milk to have a vanillin flavor which was detected by each of the three judges rating the milk. One ounce (29 ml.) of vanillin per orum caused no flavor change that could be detected by any of the three judges while 58 ml. of orally-administered vanillin caused a slight vanilla flavor which was detected by each of the three judges. As only a small fraction of the vanillin could have been inhaled, this compound appears to be more effective in causing milk flavors when inhaled than when ingested.

*Synthetic Orchid.* In only one trial with one cow, 58 ml. of synthetic orchid-alcohol solution were atomized into the "tent." None of the three judges could detect any off-flavor in the milk.

*Onions.* One half bushel of crushed onions in the manger in one trial with two cows produced milk in which all of the four judges detected a slightly objectionable or feed flavor. In a second trial with one cow, one judge pronounced the milk normal and three detected a "feed," "sweetish" and "slightly off" flavor respectively. When onions were administered orally either in 28-gram or 454-gram quantities, all judges criticized the milks as having a strong onion flavor.

*Garlic.* Garlic, in the bulbous form, was tried in one experiment only, in which case it was placed in the manger in the crushed form. Three judges declared the milk to be normal while one detected a slight feed flavor which he also observed in the normal mixed milk. These results are not in agreement with those obtained by Babcock (1) who was able to detect garlic odor in the milk drawn one minute after a ten-minute inhalation period. The cause of this discrepancy is unknown but it should be noted that Babcock used wild garlic tops while we used crushed garlic bulbs. There may be a sufficient difference in the volatility of the aromatic compounds contained in these two forms of garlic to explain this disagreement.

Our failure to demonstrate typical garlic and also onion odors in milk by inhalation would immediately suggest an inability (or insensitiveness) on the part of judges to recognize these flavors or a low odor concentration in the inhalation tent. The latter seems unlikely in view of the fact that both authors were distinctly aware of strong onion and garlic odors during the inhalation period of these trials.

*Manure.* Decomposing cow manure was used as the source of odors in two trials involving two cows each. In the first trial, three out of four judges detected "cowy" and "objectionable" flavors in the milk while in the second trial two out of three judges pronounced the milk "cowy" or "unclean." That the flavors were not pronounced is indicated by the fact that one judge in each trial called the milks "normal." However, the other com-

ments indicate that odors of decomposing manure are absorbed through the lungs and find their way into the milk. It is also possible that the effects may be more pronounced after a longer exposure than the routine two hours of these experiments.

*Corn Silage.* The milk from two cows allowed to inhale the odors from normal corn silage was pronounced to have feed flavors by three judges and to be normal by one judge.

*Alfalfa Silage.* Two cows exposed to good quality alfalfa silage produced milk which was judged as having a feed or unclean flavor by three out of four judges. When subjected to alfalfa silage with a strong odor, two cows produced milk which was found by all of the three judges to have a decided feed flavor.

*Scrapings from Roquefort Cheese.* In one trial with two cows, the odoriferous, slimy scrapings from Roquefort cheese produced no noticeable effect upon the milk as all three judges pronounced the milks to be normal.

From the foregoing, it is obvious that some inhaled substances are readily absorbed into the blood and from there pass into the milk. The lung membranes are less permeable to other substances and for this reason, the flavor of the milk is affected to a lesser extent. Then, too, the lung membranes may be highly permeable to some substances, but the udder membranes may be less permeable or even impermeable to these same materials.

Although it is not of consequence in these experiments, the point should be mentioned that exogenous flavors and odors could reasonably pass out of the milk, back into the lungs and eventually be lost to the outside air. That this apparently happens in the cow is amply demonstrated by the fact that feeding silages, etc. four or five hours before milking serves to prevent these feed flavors from occurring in the milk. The mechanism by which the above loss of flavor occurs probably involves flavor or odor concentrations between the milk, blood and lungs. When that of the lung becomes low, as it should with increasing time, the odor equilibrium is shifted toward the lung end. The loss of the odor or flavor to the outside air is similar to removing the product of a chemical reaction and allowing the reaction to go to completion. The net result is a pulling out by the lung of the odor from the milk via the blood until the odor or flavor remaining in the milk becomes unnoticeable.

Among the inhaled pure substances that cause decided flavors in the milk are turpentine, paradichlorobenzene, benzaldehyde, camphor and vanillin. All of these compounds, it should be pointed out, are ether soluble and water insoluble. These properties indicate that the above compounds are probably fat soluble and this is, no doubt, a partial explanation of the readiness with which they pass into the milk via the blood.

Of the compounds just mentioned, all except benzaldehyde impart their characteristic flavor to the milk. A reasonable explanation for the change in flavor of benzaldehyde may lie in a change in the chemical configuration

of this substance as it passes through the animal system. It is not improbable that other ingested or inhaled substances may also be changed in the animal's body so that their flavoring properties are either lost or so altered that they are not recognized.

The failure of the highly volatile and aromatic synthetic orchid compound to be detected in milk after inhalation cannot be explained without further study. This compound may not have been absorbed through the lung, or it may have been absorbed and altered so as to lose its flavoring properties or the mammary gland membranes may be simply impermeable to it.

With regard to the onion and garlic experiments, it is the opinion of the authors that the lung is not permeable to the characteristic flavors of onion and garlic but is permeable to some other flavoring constituent. That the characteristic flavoring compounds, principally allyl sulfide, of onion and garlic are permeable to the mammary gland and are not altered in the system is evidenced by the effects of oral administration of both of these products.

While the results from the inhalation of odors from decomposing manure and silage are not as striking as those from the inhalation of some other compounds, there is unmistakable evidence that they do produce "off flavors" in the milk. This would emphasize the need for keeping the cows in an atmosphere free from undesirable odors before milking.

#### SUMMARY AND CONCLUSIONS

1. The effects of inhaling the odors from thirteen substances and ingestion of five substances upon the flavor of milk are reported.
2. Inhalation of turpentine, paradichlorbenzene, camphor or vanillin caused flavoring of the milk characteristic of each of these compounds.
3. Inhalation of benzaldehyde, onions and garlic caused a change in the flavor of the milk which was not characteristic of the compound.
4. Inhalation of odors from corn silage, alfalfa silage and decomposing manure, produced "off flavors" in the milk.
5. Inhalation of synthetic orchid or scrapings of Roquefort cheese produced no detectable "off flavor" in milk.

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# MILK LIPASE AND MILK FLAVOR

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Milk flavor is a composite property (1, 5). Ultimately all its components must be referable to the individual chemical constituents of milk. In this communication evidence of a relationship of milk lipase to milk flavor is submitted.

Milk lipase brings about the hydrolysis of butterfat glycerides. The liberated fatty acids have the property of lowering the surface tension of milk. Surface tension measurement may, therefore, be used as a convenient method of following milk lipase activity (4). The free fatty acids, however, contribute also to the lowering of the flavor score of milk. They alter the taste and impart a strongly cowy, unclean, or butyric odor to the milk. Since the odor of the volatile fatty acids is fairly readily detected it was decided to utilize this property in a study of the relationship between surface tension and flavor of milk.

Lipolysis can be demonstrated in all raw milk under specified conditions (2). In susceptible milks lipolysis may take place under the usual conditions of handling milk. It follows therefore as a logical possibility that milk lipase may also affect, although to a smaller degree, the flavor of "average" milk under "average" conditions.

An interesting relationship between the decline in milk flavor and the increase in butterfat content of milk held overnight at 5° C. has been reported (5). The question arises whether this effect might not be due to the action of milk lipase.

The problem of the relation of milk lipase to milk flavor is important not only in considering milk as such but also in the study of the flavor of other dairy products, particularly perhaps of raw milk cheddar cheese (3).

## EXPERIMENTAL

A total of 144 samples of milk included not more than 4 samples from any individual cow and represented 51 cows from October 14 to December 24, during which time the cows were stabled and stall fed.

Twelve was selected as a convenient number of samples which could be satisfactorily handled during a given day. Accordingly this number of wide-mouth glass-stoppered bottles of about 100 ml. capacity was prepared by washing, treating with chromic-sulfuric mixture and finally steaming. Milk samples were then collected during the evening milking from each pail

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as it was being emptied into the milk can. The bottles were stoppered and the samples stored in a refrigerator at 5° C. overnight.

In the morning the milk samples were brought to 25° C. and after shaking to mix in the cream layer, surface tension was determined by means of the du Noüy tensiometer. Triplicate determinations on 3 portions of each milk were used to obtain an individual result. The platinum-iridium ring was flamed before each determination and a small platinum dish which was also kept clean by flaming served as a container. The results for the 12 samples were then arranged into 3 groups of 4 each with those having the lowest surface tension reading in the first group, those having the highest reading in the third group, and the remainder in the intermediate group. In case any two samples tied for the same group placing, allocation was made by lottery.

In the afternoon the remaining portion of each milk which was not used for surface tension measurement was judged at 25° C. for odor only by 3 judges. The samples were again arranged into 3 groups of 4 as previously, but this time according to odor.

The above procedure was repeated 12 times giving a total of 144 samples. The group placings of each sample according to surface tension and according to odor were then correlated by the usual statistical method, giving a correlation coefficient of 0.23.

#### DISCUSSION

The volatile fatty acids constitute only one component of milk odor (1,5). Therefore, when milks are judged on the basis of odor other normal components, feed and physiological factors are included. Since these other components are not necessarily related to surface tension, a high correlation coefficient between surface tension and odor cannot be expected.

Again in unselected milk samples a wide variation in properties cannot be expected for all samples. According to normal distribution the majority of milks will be very similar in properties and will be difficult to distinguish from one another either on the basis of surface tension or odor. Similarly the comparison of absolute values of surface tension instead of surface tension lowering is based on the assumption that the surface tension of freshly drawn milks is fairly constant and that any error from this source is small in comparison with the variations obtained among different milks. A certain proportion of the samples may therefore be assigned their group placings on the basis of random distribution rather than on physico-chemical or organoleptic criteria.

Although it is not possible to obviate all the experimental difficulties, they can be minimized. The following expedients were used. Twelve samples were used for each run. Milks were compared organoleptically relative to one another instead of on an absolute basis. They were divided into only 3 groups. The average result of 3 judges' opinions was used.

The interpretation of our experimental result, namely, a correlation coefficient of 0.23 relating the surface tension and milk odor may now be considered. The coefficient is not high, but some of the reasons for this have already been discussed. It might be pointed out by way of comparison that the same value of  $r$  was obtained by Weaver (5) in correlating milk flavor with butterfat content of milk. According to statistical tables a value of  $r=0.23$  for 144 samples is definitely significant. It may be concluded, therefore, that milk having a low surface tension after holding at 5° C. will, other things being equal, have a less desirable odor and conversely. Since surface tension is related to lipase activity and odor is a component of flavor, therefore, the flavor of milk is related to its lipase activity.

The magnitude of the correlation coefficient may be interpreted in one of two ways. It may be assumed that a certain proportion of the samples was rancid or slightly rancid. None of the milks, however, was criticized by the judges for this defect. On the other hand, volatile fatty acids may have been present in a large proportion of the milk samples, but due to other variables many of these could not be detected. In other words, if it were possible to select samples in such a way that all factors were constant except for the variables under consideration, a much higher correlation would be expected. This is probably a more accurate interpretation. One is therefore inclined to believe the presence of a small amount of free fatty acids and slight lipolytic activity in normal milk. This is particularly significant because milks used in our investigation were kept under conditions which approximate those in ordinary farm practice.

In our study on the flavor of cheddar cheese the hypothesis was advanced that many of the less definite defects, such as unclean, etc., were probably related to the rancid flavor and were caused by traces of milk lipase (3). Evidence for a parallel condition in milk flavors exists. Thus from the statements by Davis (1) and by Weaver (5) the flavor of freshly drawn milk from a healthy udder may be described as mildly and pleasantly cowy. This appears to develop into a strongly cowy flavor and finally the latter is considered as a forerunner of the rancid flavor. This sequence and relationship of milk flavors find support in our results on the study of the relation of milk lipase to milk flavor in normal milk.

#### SUMMARY

The relation between lipase activity as indicated by surface tension measurement and flavor as judged by odor was studied. The results on a total of 144 milk samples including not more than 4 samples from one cow and representing 51 individual cows, gave a correlation coefficient of 0.23. The role of milk lipase in average milk under ordinary methods of handling is discussed in relation to the flavor of milk and milk products.

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# INCREASED MILK AND MILK FAT PRODUCTION FOLLOWING THE FEEDING OF ARTIFICIALLY FORMED THYROPROTEIN (THYROLACTIN)<sup>1</sup>

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In an earlier communication, the authors (8) have presented evidence demonstrating that artificially formed thyroproteins will replace the function of the thyroid gland in thyroidectomized goats. The successful formation of thyroidally active proteins by relatively simple and inexpensive methods has suggested the practical use of such substances for the stimulation of increased lactation in dairy cattle. Preliminary results on the lactation-stimulating properties of iodinated casein when fed to lactating goats were reported by Turner (10), and at that time the term "thyrolactin" was proposed for such preparations. The present report includes data on the effect of thyrolactin, administered orally over short periods of time, on milk production of goats and milk and milk fat production of lactating cows.

## EXPERIMENTAL

*Plan of the Experiment.* The thyrolactin used was iodinated under the conditions and approximately at the optimal level indicated in the report of Reineke, Williamson and Turner (9). Such preparations were found to have a potency of 1/100 to 1/200 that of synthetic thyroxine as measured by biological assay on guinea pigs and tadpoles.

In the experiments on goats, thyrolactin was given in the daily grain feed in varying amounts over a period of 5 days. Daily measurements of the pulse-rate were taken, as a rough check on the metabolic effect of the treatment. Since it was desired primarily to determine whether or not thyrolactin would affect milk production, only the daily milk weights were taken, and no milk analyses were made.

In the cow experiments, the thyrolactin was given by mixing it with the grain at the afternoon feeding for three days in succession. Since certain cows refused to eat the material given in this way, because of its unfamiliar odor, the expedient was adopted later of mixing the thyrolactin with the grain feed, and then adding a small amount of molasses to the mixture. All cows ate the feed readily when given in this manner.

In certain of the trials, pulse records were taken, and milk samples were collected for a fat analysis; in the remainder only milk production records are available.

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## RESULTS

The results of 5 trials in which 5 to 10 grams of thyrolactin were fed daily to 3 to 6 lactating goats are summarized in table 1. The results of a fourth trial in which U. S. P. desiccated thyroid was given instead of thyrolactin are included. Goat No. 836 had been lactating for about 14 months; the remainder of the animals were in the 2nd to 4th months of lactation at the beginning of the experiments.

TABLE 1

*The effect of feeding thyrolactin on the milk production and heart rate of lactating goats*

Date (1941)	Thyrolactin fed daily	Goat No.	Milk 5 days before thyrolactin feeding	Milk 5 days during thyrolactin feeding	Milk 5 days after thyrolactin feeding	Increase	Heart rate increase (beats per minute)
	<i>grams</i>		<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>%</i>	
May 7-11	10	433	19.7	20.5	21.1	+ 7.1	+ 13.5
	5	843	4.9	5.7	6.9	+ 40.8	+ 18.2
	5	304	14.7	14.2	15.2	+ 3.4	+ 5.5
June 2-6	10	433	19.0	18.0	21.0	+ 10.5	+ 6.4
	10	943	6.4	6.0	7.4	+ 15.6	+ 14.4
	10	304	14.1	14.1	15.3	+ 8.5	+ 4.0
	10	437	14.1	14.0	14.5	+ 2.8	+ 7.1
	10	836	10.0	10.4	11.1	+ 11.0	+ 2.2
July 6-10	10	433	14.7	14.9	17.8	+ 21.1	+ 10.8
	10	843	6.6	6.2	6.8	+ 3.2	+ 8.8
	10	304	12.0	11.3	12.1	+ 0.8	+ 3.1
	10	437	12.7	12.8	13.6	+ 7.1	+ 1.0
	10	443	9.2	9.4	9.7	+ 5.4	+ 8.2
	10	836	10.2	10.7	11.2	+ 9.8	+ 12.0
						+ 10.51*	+ 8.2*
July 25-29 (des. thy- roid)	10	433	16.4	16.0	16.7	- 1.8	- 3.0
	10	843	6.0	6.0	5.9	- 1.7	- 6.0
	10	304	11.6	12.3	10.5	- 9.5	- 4.0
	10	437	12.3	11.1	14.0	+ 12.1	- 9.0
	10	836	10.7	9.9	9.9	- 7.5	- 5.0

No. 836 had been lactating for more than a year. All other goats were in the second to fourth months of lactation.

\* Mean.

It will be noted that the extent to which milk production was stimulated was quite variable, but there was a significant increase in milk yield in most cases. Comparisons of the milk yield for the five days preceding the thyrolactin feeding with the 5-day yield subsequent to the feeding period show increases in production ranging from 0.8 to 40.8 per cent, with an average increase of 10.51 per cent. There were also increases in the heart rate ranging from 1.0 to 18.2 beats per minute and averaging 8.2 beats per minute.

Five of the goats that had previously been used for the thyrolactin experiments were given a rest of two weeks, and then were fed U. S. P. desic-

cated thyroid at the rate of 10 grams daily. One goat responded with an increase of 12.1 per cent in milk production; two goats showed no change; and production of the remaining animals decreased 9.5 and 7.5 per cent, respectively. The heart rates actually showed a slight decrease. It is believed, however, that this is not due to any depressing effect of the thyroid substance, but that the decline in pulse rate expresses the response to a change from high environmental temperatures at the start of the experiment to more nearly normal levels in the later stages. Thus the stimulating effect of thyroid substance on the heart rate was probably masked by environmental factors that showed an opposite trend. While the number of trials with desiccated thyroid is probably too small to warrant a final conclusion, it appears that thyrolactin has a greater effect on lactation than the particular samples of desiccated thyroid used. Extensive assays on guinea pigs have also indicated that the better preparations of thyrolactin have somewhat greater potency than this lot of desiccated thyroid. Since U. S. P. desiccated thyroid is standardized by its iodine content, and not by biological assay, it cannot be predicted whether or not this relationship would hold with other lots of thyroid substance.

The results of feeding thyrolactin in a total of 14 trials with nine different cows are summarized in table 2. The cows used in these experiments were in the 4th to 10th months of lactation, at which time, as shown by previous work with thyroid substance and thyroxine, they should be responsive to thyroidal stimulation. The thyrolactin was given only during a 3-day period, and the effects are measured by comparing the 3-day production preceding the feeding period to the 3-day production subsequent to the 3rd day of thyrolactin feeding. It will be noted that there was a slight rise in production during the feeding period. This is due to the fact (shown by the individual data) that evidences of increased production appear by the third day after thyrolactin feeding is begun. During the 3-day period following treatment with thyrolactin, there was a significant rise in milk production in all except two cases, ranging from 6.09 to 22.6 per cent and averaging 8.59 per cent.

Unfortunately, it was not feasible to collect milk samples for analysis in all of the experiments. However, in the six trials in which milk fat analyses were made, there were pronounced rises in fat test in two cases and no appreciable change in the other four. The trials of Experiment II were made during the very hot weather of July at a time when normal fat tests fluctuated considerably in most cases, making comparisons difficult. Despite this fact it is believed that the 16.5 per cent rise in fat test and increase in fat yield of 26.8 per cent for cow No. 750 is significant, since in this case the milk fat percentage showed a steady and gradual rise as a result of stimulation with thyrolactin.

Cow No. 762 showed an increase of only 1.62 per cent in milk production,



TABLE 2

*The effect of feeding thyrolactin on milk production, milk fat percentage, and milk fat production of dairy cows*

Cow No.	Month of lactation	Milk production			Average milk fat percentage			Milk fat production			Remarks
		3 days before thyrolactin feeding	3 days during thyrolactin feeding	3 days after thyrolactin feeding	Gain or loss	3 days before thyrolactin feeding	3 days during thyrolactin feeding	3 days after thyrolactin feeding	Gain or loss	3 days before thyrolactin feeding	
		<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	%				%		
Experiment I (run in May; 100 grams of thyrolactin from lot 36 fed daily)											
762H	4th	153.1	159.2	155.6	+ 1.62	3.26	3.72	4.13	+ 26.7	5.001	6.428 + 28.50
Experiment II (run in July; 100 grams of thyrolactin from lot SS fed daily)											
750H	4th	121.1	125.0	131.5	+ 7.90	3.15	3.13	3.67	+ 16.5	3.814	4.838 + 26.84
455G	6th	50.6	50.9	54.3	+ 6.72	5.21	5.15	5.22		2.715	2.915 + 7.36
926J	5th	68.3	68.1	73.1	+ 8.49	5.71	5.12	5.86	+ 2.63	3.920	4.286 + 9.34
921J	5th	58.6	60.3	63.6	+ 8.53	4.83	4.63	4.68	- 3.08	2.832	2.984 + 5.37
857J	6th	92.0	89.9	99.6	+ 8.26	4.73	4.78	4.63	- 2.12	4.953	4.615 + 6.02
Experiment III (run in September; 100 grams of thyrolactin fed daily)											
750H	6th	97.8	97.8	105.1	+ 7.46						
455G	8th	34.5	34.1	36.6	+ 6.09						
926J	7th	41.6	45.1	45.3	+ 8.89						
921J	7th	39.4	40.3	44.4	+ 12.69						
857J	8th	51.8	48.4	52.4	+ 00.11						
Experiment IV (run in February; 100, 75, and 50 grams respectively of thyrolactin from lot 36 fed daily)											
869J	6th	36.6	39.9	40.7	+ 11.20						
923J	10th	38.9	40.8	47.7	+ 22.60						
738H	7th	100.0	100.8	109.7	+ 9.70						
Mean					+ 8.59				+ 6.77		+ 13.90

but her fat test increased 26.7 per cent, resulting in an increase of total fat yield of 28.5 per cent. So far as is known, no unusual environmental factors came in to influence this result.

The average daily milk production obtained in the 14 individual trials included in this report as well as the average heart rate in four trials is pictured in figure 1. Milk production began to rise on the third day of thyrolactin feeding, and then, even though no further thyrolactin was given, continued to rise for three days more. This was followed by a gradual decline in production to somewhat below the base level as the effects of the stimulation wore off. Even though heart rates were obtained on only four

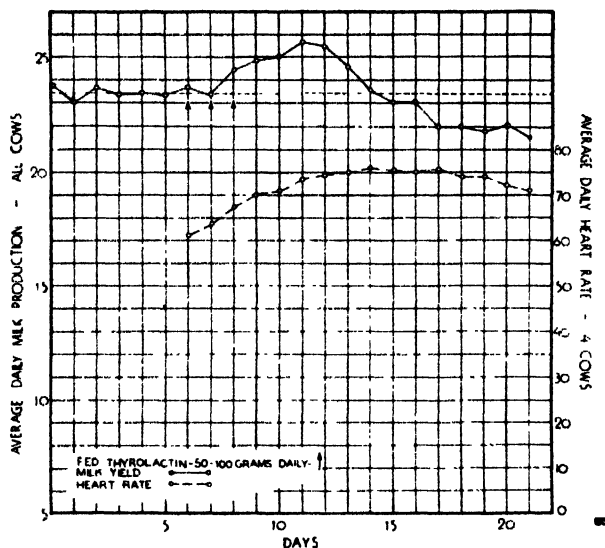


FIG. 1. The effect of feeding thyrolactin for a 3-day period on the milk production and heart rate of lactating cows. Thyrolactin was fed at the rate of 50 to 100 grams daily per cow.

cows, the average of these values shows a very uniform trend, rising parallel with the milk yield. The heart rate was maintained at an elevated level for about two weeks, whereas milk production dropped to normal within a week after the thyrolactin was discontinued.

#### DISCUSSION

The experiments reported herein clearly demonstrate that optimally iodinated milk proteins will stimulate increased milk production when fed for short periods to lactating cows and goats. When thyrolactin was fed to goats at the rate of 5 to 10 grams daily for a period of five days, there was an average increase in milk production of 10.5 per cent. The feeding of 50 to 100 grams of thyrolactin daily in 14 individual trials on nine different cows resulted in an average increase in milk production of 8.59 per cent.

In the six cases in which fat tests were obtained on the milk there was an average increase of 6.77 per cent in fat test and 13.9 per cent in fat yield. From these results it appears warranted to conclude that insofar as the stimulation of milk production is concerned, thyrolactin has all of the properties of desiccated thyroid or thyroxine as first reported by Graham (2, 3) and confirmed and extended by Jack and Bechdel (6), Folley and White (1), Herman, Graham and Turner (4), and Ralston *et al.* (7).

The percentage increases in milk and fat production are somewhat less than reported by previous investigators following the injection of thyroxine or the feeding of desiccated thyroid. However, since the response to be obtained following thyroidal stimulation is roughly proportional to the dosage, greater increases could in all likelihood be obtained by feeding larger amounts of thyrolactin.

In view of the results obtained it appears that the feeding of thyrolactin shows considerable promise as a means of stimulating increased milk production in lactating dairy cattle. It is true that most of the experiments conducted to date on the thyroidal stimulation of lactation in dairy cattle have been run over relatively short periods of time, and so might not reflect the true effect that would be obtained under practical feeding conditions. However, it was shown by Herman *et al.* (4) that the feeding of thyroid even at the peak of lactation tended to prevent the normal decline in milk production. A second feeding period during more advanced lactation resulted in a sharp rise in both milk and fat yield. Ralston *et al.* (7) demonstrated that lactating cows will respond to the injection of thyroxine with increased yields of milk and butterfat at all stages of lactation, though the greatest responses were obtained during the period of declining production. Hurst (5) reported that when 10 to 15 mg. of thyroxine were injected daily for a period of four weeks, milk production was maintained and in some instances there were sharp increases of as much as 38 per cent in milk yield. The yield of milk fat was increased even more than milk production. The injection of thyroxine into a single cow for five months increased her persistency index from an average of 91 per cent in the first six months of five previous lactations to 95 in the first six months of the experimental lactation.

Though the present experiments might be criticized from a practical standpoint because of their short duration, it is believed on the basis of the work cited above that the beneficial effects of thyrolactin feeding could be maintained throughout the lactation period. The initial results on long time experiments which are now in progress in this laboratory (unpublished) would tend to support this view. It is probable that with continued feeding of thyrolactin the desired result can be obtained with a considerably lower dosage than that used in the present experiment.

It is not within the scope of this paper to discuss the chemical problems

concerned with the formation of thyroidally active substances. For a review of this question the reader is referred to the report of Reineke, Williamson, and Turner (9). It may be well to point out, however, that in order to produce iodoproteins with satisfactory thyroidal activity it is necessary to carry out the process within rather narrowly defined conditions. While iodine can be combined with proteins by a variety of methods, the mere fact that a protein contains organically combined iodine, even though the total iodine is present in the same amounts as in thyroidally active preparations, is no guarantee of its potency. It is recommended, therefore, that iodoproteins to be used for the stimulation of lactation be produced by well controlled methods that are proved to give satisfactory results, and further, that they be assayed biologically before being put to extensive use.

#### SUMMARY

1. Optimally iodinated milk proteins (thyrolactin) were fed to lactating goats and cows and its effect on milk production was noted.

2. When fed to goats in declining stages of lactation at the rate of 5 to 10 grams daily, thyrolactin stimulated increases in milk production ranging from 0.8 to 40.8 per cent and averaging 10.51 per cent. The heart rate was accelerated an average of 8.2 beats per minute.

3. Thyrolactin fed in amounts of 50 to 100 grams daily to nine cows in a total of 14 individual trials caused an average increase in milk yield of 8.59 per cent. The individual increases ranged from 6.09 to 22.6 per cent. In six trials in which milk fat analyses were made there was an average increase of 6.77 per cent in fat percentage and 13.9 per cent in fat yield.

4. The results are discussed in relation to previous work that has been done on the effect of thyroid feeding and thyroxine injection, and the practical possibilities are pointed out.

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# THE AVAILABILITY OF THE IRON OF COCOA AND OF IRON-FORTIFIED COCOA MIXTURES

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## INTRODUCTION

Investigation has shown (1, 12, 17) that our national diet is more frequently deficient in iron than in any other mineral nutrient. To correct this situation two procedures are possible. The first is to improve the quality of the diet by educating people to consume foods known to be good sources of available iron. Such an undertaking moves slowly, however, and is handicapped by a lack of knowledge of the degree of availability of the iron of many foods. The second, and the present tendency, is to add the needed nutrient to commonly consumed foods. Chocolate and cocoa mixtures to be used in the preparation of chocolate milk are among the list of such iron-fortified foods.

Iron-fortification of foods is not always successful since free iron salts act as catalyzers in the oxidation of fats, thereby increasing oxidative deterioration. Cocoa and chocolate contain some natural antioxidants as evidenced by the fact that they protect natural flavors against oxidation (13). Therefore, foods such as chocolate and cocoa which are not readily subject to the development of rancidity are good carriers of added iron in so far as flavor is concerned. However, the mere addition of a desirable nutrient to a substance is no assurance that the added ingredient will remain available. The presence of tannic substances and phosphorus in cocoa suggests the possibility of removal of the iron by the formation of insoluble salts, which may make chocolate and cocoa unsatisfactory as carriers of added iron.

The total iron of cocoa and chocolate may be no more than 14.3 and 3.28 milligrams per hundred grams (11) respectively. However, the consumption of these products, whether fortified with iron or not, is great enough to make them an important source of iron, provided it is all available.

Because little is known about the nutritive value of the iron in cocoa, whether natural or added, this study was undertaken.

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## GENERAL PLAN OF STUDY

The purpose of this study was accomplished by measuring the degree of hemoglobin regeneration in anemic rats when cocoa and iron-fortified cocoa were fed at the same level of iron intake as an inorganic salt of iron. An attempt was made to check the results by the chemical procedure of Hill (9), but because of color interferences, this had to be abandoned. Since comparatively little is known about the tannic substances in cocoa, their influences were studied by the use of pure tannic acid.

## EXPERIMENTAL

In determining the nutritional value of the iron of cocoa and iron-fortified cocoa a modification of the Elvehjem and Kemmerer (5) technique was followed. White rats of the Battle Creek strain were used in this experiment. Before weaning, the young rats had access only to whole milk powder (6) and distilled water in addition to the mother's milk. They were weaned at three weeks and at this time were placed in individual galvanized iron wire cages which rested on raised screening of three mesh to the inch. The rats were fed dried whole milk *ad libitum* in porcelain cups. In accordance with the recommendation of Smith and Otis (16), when the animals were twenty-eight days old, they were given 0.05 milligrams of copper as the sulphate to insure depletion of the iron stores in the liver and 0.04 milligrams of manganese as the chloride. The rats were weighed weekly and as soon as the subjective signs of a developing anemia were evident, weekly hemoglobin determinations were made. The Newcomer method with a Klett

TABLE 1  
*Feed formulas used*

Ingredient	Group I	Group II	Group III	Group IV	Group V	Group VI
	%	%	%	%	%	%
Whole milk powder ..	54.0	54.0	54.0	54.0	54.0	54.0
Cocoa ....	14.0			14.0		
Sugar ....	32.0	32.0	32.0	32.0	32.0	32.0
Casein ....		1.4	1.4		1.4	1.4
Cornstarch ....		8.7	7.0		8.7	8.7
Crisco ....		1.6	1.6		1.6	1.6
Tannic acid .....			1.7			

## Iron intake per day per rat

	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.
Iron, from cocoa ....	0.19	0.00	0.00	0.19	0.00	0.00
Iron, from ferric chloride ..	0.00	0.19	0.19	0.11	0.30	0.00
Iron, total* ....	0.19	0.19	0.19	0.30	0.30	0.00

NOTE: Each rat in all groups was fed 0.05 mgms. of copper and 0.04 mgms. of manganese per day.

\* The very small amount of iron in the whole milk powder (0.0008 per cent) is not included in the total.

colorimeter was used and the solutions were read against a standard New-comer glass disk. After approximately forty-six days, when the hemoglobin values were between 3.5 and 4.5 grams per 100 cc. of blood the rats were considered sufficiently anemic to be used for a recovery experiment. At this time the animals were distributed as evenly as possible with respect to age, weight, and hemoglobin level into six groups. All groups contained equal numbers of both sexes and consisted of twelve animals each except the negative control group which consisted of ten animals.

Six diets were compounded as shown in table 1, and fed one to each of the six groups. These diets were approximately alike in nutrients and calories, but they differed in the source and amount of iron. The cocoa powder used (table 2) was chosen after analyses of eighteen commercial

TABLE 2  
*Analyses of cocoa powder*

Constituents	Per cent
Moisture	5.40
Protein	22.62*
Theobromine	1.75
Caffeine	0.12
Fat	11.23
Fiber	5.25
Ash	5.98
Tannic substances	12.15†
Other N-free matter	35.50
Iron	0.0134‡

\* Nitrogen (minus theobromine and caffeine nitrogen)  $\times$  6.25.

† Ulrich's method as modified by Kuzmeski and Mueller (unpublished results).

‡ Per cent of iron as total iron.

powders because of the high percentage of tannic substances (12.15 per cent) present. On a fluid milk basis the cocoa-milk diet would represent 3 per cent cocoa, 7 per cent sugar, and 90 per cent milk, and approximates chocolate milk with a maximum amount of cocoa. The whole milk powder was used instead of fluid milk because of obvious advantages in feeding. Group I received this ration plus the copper and manganese solution; and Group IV the same as Group I plus ferric chloride to raise the total iron intake to a level of 0.3 milligrams daily. Ten grams of each of these two rations were fed to each rat daily.

The whole milk powder rations were made up to simulate the cocoa ration in nutritive value. Casein replaced 38 per cent of the cocoa protein since only that percentage of cocoa protein is utilized by the rat. Neutral fat, uncontaminated with iron, replaced the cocoa fat, and starch was used to make up the carbohydrate and remainder of the protein fraction. No agar nor inert material was used to replace ash and fiber since this might interfere with absorption. Since these constituents were omitted, 9.7 grams



of these rations were fed daily, this amount being equivalent in nutrients and calories to ten grams of the cocoa rations. Experimental groups II, V, and VI received this ration plus copper and manganese. Groups II and V were positive control groups receiving 0.19 and 0.3 milligrams of iron daily as ferric chloride, and Group VI was the negative control group receiving no iron supplement.

The whole milk powder ration plus tannic acid was fed to Group III, and was similar to that fed Group II, except that pure tannic acid was added and a corresponding amount of cornstarch was omitted. This ration was also fed at the 9.7 grams daily level.

All mineral salts were fed in solution. During the depletion period, after the animals were twenty-eight days old, the copper and manganese solution was fed by pipette daily, six days per week. After the depletion period the mineral solutions were fed three times each week and were pipetted onto a small amount of the dry ration in a glass dish. The animals consumed it greedily the moment it was placed in the cage.

Weekly hemoglobin determinations and weighings were made throughout the experimental period which lasted for six weeks. During this time all animals were kept on an equivalent food intake. Preliminary investigation showed that approximately ten grams daily fed six days a week was the maximum which the animals could consume at the age when they were put on the experiment. Therefore, 10 and 9.7 grams per day, as explained in the discussion of the rations, were fed six days per week until the animals were seventy days old; after which time a seventh 10- or 9.7-gram portion of the basal ration was added weekly until the rats were sacrificed. This food allotment allowed substantial gains through the six-week period. Constant care was taken that all food was eaten and all animals which failed to consume the allotted portion were discarded. All animals continued to receive both copper and manganese during the experimental period; the copper to insure utilization of iron (7), and the manganese because investigation has shown that rats will thrive on a milk diet only if supplemented with copper, iron, and manganese (8).

#### RESULTS AND DISCUSSION

The average hemoglobin responses and weight gains of all groups during the six-week experimental period are given in table 3. Both males and females are included in the averages because sexes were equally divided and also because the separate analysis of data showed no significant difference between sexes. Results indicate that the iron of cocoa is not so well utilized as an equivalent amount of iron fed as ferric chloride. Group I, receiving cocoa as a source of iron regenerated 5.3 grams of hemoglobin per 100 cc. of blood; while Group II, receiving the same amount of iron as ferric chloride regenerated 8.4 grams during the six weeks of the experimental

period. Thus, the iron of cocoa regenerated approximately two-thirds as much hemoglobin as an equivalent amount of ferric chloride. The fact that Group III regenerated the same amount of hemoglobin as Group II, indicates that the addition of tannic acid did not decrease the utilization of iron. In fact the rapid regeneration of hemoglobin in this group during the first three weeks of the experiment might indicate that the presence of the tannic acid favored the rapid absorption of iron during the early part of the period (3). It may be that the water-insoluble iron tannate was made utilizable by gradual solution in the digestive fluids (2).

TABLE 3

*The average age, weight, and hemoglobin regeneration of six experimental groups of anemic rats*

Group No.	Ration fed*	Iron in ration*	Initial age	Initial weight	Weight after 6 weeks	Gain in weight	Hemoglobin in grams per 100 cc. blood		
							Initial	After 6 weeks	Total gain
I	Milk plus cocoa	<i>mgms.</i> 0.19	<i>days</i> 46	<i>gms.</i> 85	<i>gms.</i> 137	<i>gms.</i> 52	3.8	9.1	5.3
II	Milk plus iron	0.19	46	90	161	71	3.8	12.2	8.4
III	Milk plus iron plus tannin	0.19	49	99	158	59	4.2	12.5	8.3
IV	Milk plus cocoa plus iron	0.30	48	88	133	45	3.9	12.2	8.3
V	Milk plus iron	0.30	46	91	158	67	4.0	13.3	9.3
VI	Milk	0.00	46	87	145	58	4.0	5.4	1.4

\* For complete information see table 2.

The results show that when the cocoa-milk ration was fortified with iron, the added iron was just as available in the presence of cocoa as when added to a whole milk ration. Addition of 0.11 milligrams of iron daily, which increased the daily intake to 0.3 milligrams, permitted Group IV to regenerate 8.3 grams of hemoglobin per 100 cc. of blood as compared with 5.3 grams when cocoa was not fortified. Group V, receiving the same level of iron as Group IV, but all of it in the form of an iron salt added to the milk ration, regenerated 9.3 grams of hemoglobin. This is an increase of 1.0 gram over that produced on an intake of 0.19 milligrams of iron. The difference in these gains is in the order of magnitude to be expected on the basis of utilization of cocoa iron observed for Groups I and II. The hemoglobin gain of Group IV receiving cocoa plus ferric chloride is almost identical with that of Group II, receiving 0.19 milligrams of iron as the ferric

salt daily. Therefore, it may be concluded that Group IV must have received an equivalent amount of utilizable iron; that is, 0.11 milligrams derived from the inorganic salt, the remainder from the cocoa.

The fact that an addition of 0.11 milligram of iron daily produced an increment of gain in hemoglobin of 3.0 grams in Group IV and of only one gram in Group V may seem inconsistent. This difference may be explained, however, by the fact that there is a lowering in the degree of efficiency with which the animal body utilizes iron as the amount of available iron increases. Hemoglobin gains are greater in proportion to the amount of iron fed at the lower levels of iron intake (15). In Group II the level of iron before enrichment was nearly sufficient for complete regeneration (4). However, because the iron of cocoa is not as well utilized as an equivalent amount of inorganic iron and the level fed before enrichment was below that necessary for complete regeneration, the added increment was used more efficiently. Thus it is possible to conclude that the added iron was all utilized.

Groups II and V had average weight gains of 71 and 67 grams during the six weeks of the experimental period. The four-gram difference is insignificant. However, Groups I and IV, receiving the cocoa rations, and Group III, receiving the tannin ration gained but 52, 45, and 59 grams respectively during the same period. Negative controls (Group VI) grew slightly better even than those receiving cocoa, indicating that cocoa is detrimental. These findings are in line with those of other workers. Lease and Mitchell (10) found that levels of tannic acid greater than five per cent retarded rate of growth of white rats. Mueller and Ritchie (14) report that four per cent of cocoa in a mineralized milk ration definitely retarded growth in white rats. However, since the animals used in this study were anemic, and food intake was restricted, growth data are not as striking as they might be under other circumstances. It was also noted that hard, dry feces appeared in all rats soon after cocoa feeding was started in spite of the usual tendency to diarrhea on milk rations.

The effect of the tannic substances in cocoa on the availability of iron in cocoa is not conclusively determined by this study. Tannin added in the form of tannic acid did not reduce the availability of iron in a milk diet. It should be pointed out that tannic acid may not have the same effect on the utilization of iron as the tannic substances which are present in cocoa, about which little is known. The nature of the iron-containing compounds in cocoa needs to be determined before it can be stated that any specific substance is responsible for reducing the availability of cocoa iron to the animal body. The tannins of cocoa do not appear, however, to reduce the availability of iron added to enrich the cocoa mixture. It appears that foods containing cocoa and chocolate are well suited to be fortified with iron: first, because the added iron remains completely available; and second, because

cocoa contains some natural antioxidants which retard or prevent the oxidation of the fat in the presence of catalyzing iron salts.

#### SUMMARY AND CONCLUSIONS

1. The availability of the iron of cocoa and iron-fortified cocoa has been determined by biological procedure.

2. The iron of cocoa regenerated approximately two-thirds as much hemoglobin as an equivalent amount of ferric chloride.

3. Approximately two (1.7) per cent of pure tannic acid did not reduce the availability of the iron added to a milk ration.

4. Iron added to a cocoa mixture was completely available, indicating that the factor which limited the availability of the iron of cocoa had no influence on added iron.

5. The fortification of cocoa or chocolate milk with iron may be warranted on the basis of the availability of the added iron and on the antioxidants which retard or prevent rancidity development in the presence of iron.

6. The indiscriminate use of chocolate and cocoa in milk is not recommended because of the yet unexplained effect of cocoa on growth and intestinal function.

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## THE CURD NUMBER TEST. A METHOD OF TESTING THE CURDLING QUALITIES OF MILK\*

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Cow's milk coagulates when taken into the human stomach. Small soft curds such as those formed from boiled milk are considered to throw less of a strain upon digestive functions and to be more readily tolerated than are the large bulky curds of raw or pasteurized milk (1, 3, 4). For investigation of the curdling properties of milk the laboratory technique most widely used at this time is the curd tension test (7, 9), which measures the toughness of the clot which forms when milk is made to coagulate under certain prescribed conditions. In this test the only variable permitted lies in the milk itself, the framework of test conditions being frozen into a rigid routine, and deviations from the prescribed directions yield undependable results. The present paper records: a fresh approach to the investigation of coagulation of milk in the human stomach by presenting a device for reproducing more closely the actual happenings within the stomach than does the curd tension test; a standardized procedure for preserving and measuring the curds which form; an empirically derived scale for indicating the curd size distribution and discussion of the results obtained. This technique utilizes the apparatus originally developed by Chambers and Wolman (2), but with an improved routine of operation and a new system for calculating index numbers or "curd numbers." The successive steps which led to the crystallizing of the test procedure into its final somewhat arbitrary form and the reasoning which lies behind the more important features are included in the presentation.

### THE CURD NUMBER TEST

*Apparatus:* The curdling of the milk takes place within rubber bags which hang in a water bath (fig. 1). This bath measures  $30 \times 20 \times 10$  inches, and is lined with copper. Six inches of water, maintained at a temperature  $37^{\circ}$  and  $37.5^{\circ}$  C. by a thermostat-controlled heat coil are kept in it during operations. Across the top of the tank run two bars studded at equidistant intervals with metal clips that hold short tubular glass cylinders from which the bags are suspended. These cylinders, open at both ends, measure 2 cm. in diameter and 7 cm. in length and are made with a rimlike lip at the lower end. The bags are of thin rubber latex (fig. 2). One and one-half inches above the floor of the tank hangs a tray-like platform divided into slotted

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troughs by copper strips. Each trough measures  $8 \times 24$  in., and receives the rounded tip of a milk-filled bag. The platform is suspended at each end by a center pivot connected by rocker arms to an eccentric gear driven by an electric motor. When the machine is in operation the platform rocks back and forth about 30–40 times per minute, and imparts gentle rhythmic agitation to the contents of the bags, the rate of oscillation of the platform being controlled by an adjustable rheostat.

*Operation:* At the start of an experiment the open mouths of a series of bags are fastened by rubber bands to the lower ends of the glass cylinders.

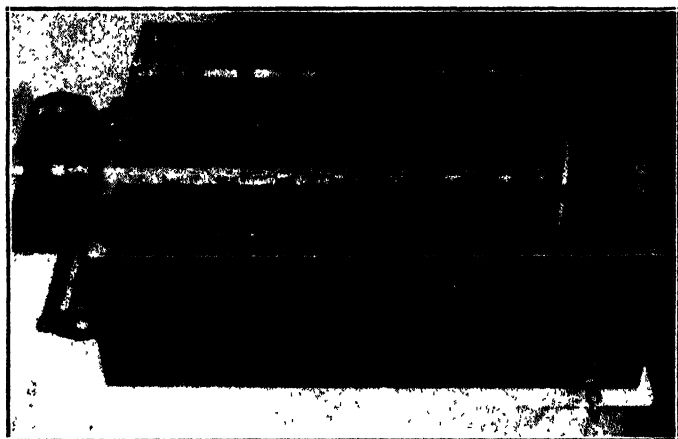


FIG. 1. Apparatus used in the artificial coagulation of milk preparations. Note the water level, the slotted tray at the bottom, the rheostat-controlled motor and the peripheral heating coil. The spigot at the lower end is for emptying the tank.

Nine bags are filled with each type of milk being tested. One hundred ml. of milk are placed in each, and the height of the cylinder adjusted so that the fluid levels inside and out coincide without undue tension being thrown on the rubber walls of their submerged portions. Generally speaking, the bags have about four-fifths of their substance submerged. The empty upper fifth collapses and lessens surface evaporation. When the temperature of the contained milk has risen to that of the water bath, the platform is set in motion. A measured amount of coagulating solution (2–4 ml. N/1 hydrochloric acid, as much as is necessary to produce the desired pH, as shown by a preliminary titration, plus 3 ml. of a solution of 0.6 per cent commercial pepsin powder U.S.P.) is introduced by gravity from a pipette. Coagulation of the milk is observed to take place within the first few minutes. Agitation of the bags is continued for thirty minutes. At fifteen minutes they are shaken for a moment in order to assure uniform distribution of the coagulant. The bags are next emptied in groups of three into 16-oz. glass jars containing 30 ml. of formalin (40 per cent formaldehyde solution), or

individually into 4-oz. jars containing 10 ml. of formalin. After being hardened from 24 to 72 hours, or indefinitely longer, the jar contents are washed through a battery of three 8-inch sieves, of mesh sizes  $1/2$  in.,  $1/10$  in. and  $1/100$  in., by means of a stream of flowing tap water. The heaps of curds thus selectively screened are transferred to previously weighed filter papers, and spread out to dry on wire screens at room temperature (fig. 3). After

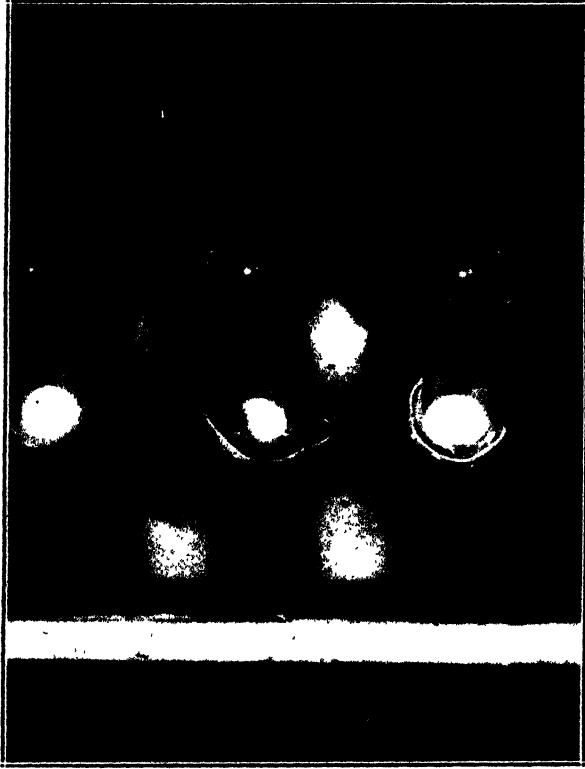


FIG. 2. Close-up of the glass cylinders to which are attached the filled rubber bags. The milk has not yet coagulated. The bags are rhythmically flexed as the supporting platform rocks to and fro.

two or three days the heaps of dried curds are then weighed individually to 0.01 gm.

*Calculation:* The following formula is then applied:  $1a + 2b + 3c = \text{curd number}$ . ( $a$  = Percentage of total curd weight caught by the  $1/2$  in. sieve;  $b$  = Percentage of total curd weight caught by the  $1/10$  in. sieve;  $c$  = Percentage of total curd weight caught by  $1/100$  in. sieve.)

#### DERIVATION OF CONCEPT OF CURD NUMBER

The early studies on curd size measurement (2) employed 8 graded sieves of mesh sizes 1 in.,  $1/2$  in.,  $1/4$  in.,  $1/6$  in.,  $1/10$  in.,  $1/20$  in. and  $1/100$  in.



Commercially such sieves are referred to in terms of their reciprocals, as No. 1, No. 2, etc. Raw and pasteurized milks would yield large curds, to be caught by series No. 1 and No. 2, whereas with specimens of boiled or homogenized milk the first sieve to trap any curds would be No. 4, No. 6 or No. 10.

The original recommendation (2) was based upon the assumption that peptic digestion is a peripheral process, taking place only at the curd surface, and that for any given specimen of milk the speed of digestion by proteolytic enzyme is proportional to the total surface area of the curds. The

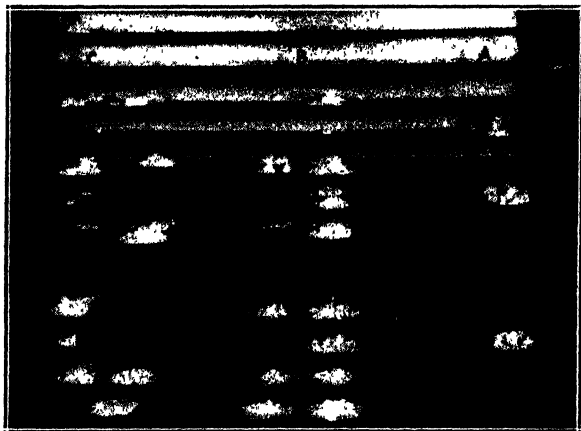


FIG. 3. Photograph of curds being dried on wire racks. Each horizontal row represents curds collected from 300 cc. of milk. Column A shows the large-sized curds caught by the 1/2 in. sieve, column B the medium-sized curds caught by the 1/10 in. sieve, and column C the masses of fine curds caught by the 1/100 in. sieve. The "abc" milks depicted here are raw and pasteurized samples, whereas the "bc" types shown are assorted homogenized and boiled preparations. (See text.)

total surface area<sup>1</sup> expressed in square centimeters or similar units might thus be taken as an expression of the availability for digestion of the milk in question. In such a system of calculation several additional assumptions became necessary. All curds were considered to be solid, spherical and smooth-surfaced, the measured breadth of each individual sieve opening was taken as

<sup>1</sup> The formula was derived as follows:

$$\begin{aligned}
 &\text{volume} \times \text{specific gravity} = \text{weight} \\
 &\text{volume} \times 3/r = \text{surface area} \\
 &\frac{\text{weight}}{\text{specific gravity}} \times 3/r = \text{surface area} \\
 A = \frac{100 \times 3}{a \times 1.2} \left( \frac{a_1}{r_1} + \frac{a_2}{r_2} + \dots + \frac{a_n}{r_n} \right) = \frac{250}{a} \left( \frac{a_1}{r_1} + \frac{a_2}{r_2} + \dots + \frac{a_n}{r_n} \right) \\
 &A = \text{Total surface area of 100 gm. of curds.} \\
 &a = \text{Total curd weight.} \\
 &1.2 = \text{Approximate specific gravity of milk curds.} \\
 &a_1, a_2, \dots, a_n = \text{Total weight of curds from each sieve.} \\
 &r_1, r_2, \dots, r_n = \text{Radius of curds from each sieve.}
 \end{aligned}$$

the true measure of diameter, and all curds caught on any one sieve were assumed to be of the same diameter. The surface area of 1 gm. of fine curds caught on the No. 100 (1/100-in. mesh) sieve would calculate out as being 10 times greater than that of 1 gm. of curd trapped on the No. 10 (1/10-in. mesh) sieve, and 100 times greater than the same amount caught on the No. 1 (1-in. mesh) sieve.

The practicability of total curd surface area as an indicator of curd size was tested out thoroughly, being computed in hundreds of determinations. This system of computation not only proved involved, slow and cumbersome, but failed on many occasions to give consistently reproducible results with identical specimens of milk.

Minor fluctuations in the weight of curds on the finest sieves—deviations within the range of error of the experiment—would exert a disproportionate effect on the total value computed for the milk. Thus, for example, a milk sample might yield one or two large coagula unable to pass the No. 2 sieves but have the remaining curds so small as to be caught only by the No. 40 and No. 100 sieves; for this milk the total surface number by virtue of the great number of small particles would calculate as high or even higher than would a different milk specimen which yielded a mass of medium-sized curds of more or less uniform size. This was probably the reason why, when comparing milks one with another, the curd surface area figures often failed to show any consistent correlation with the curd tension values for the same milks. Other workers (10, 11) have recently reported similar discrepancies.

After exploring other possible systems of computation with equal lack of success, the problem of providing a sound practical index for the curdling behavior of milk was approached from the angle of *curd size* alone. The absolute number of sieves was first reduced from eight to three in order to simplify the manipulations and calculations. Thus the No. 1 sieve was eliminated and all curds caught by No. 2 were considered "large." The No. 4 and No. 6 sieves were removed and all curds which would pass No. 2 but were caught by No. 10 were considered "medium-sized." The No. 20 and No. 40 sieves were also dropped and No. 100 was retained to catch those "fine" curds which passed sieve No. 10. Then, by referring to the large curds as "a," to the medium-sized as above described as "b" and to the fine ones as "c," it was possible to establish a crude but practical system for characterizing the curds formed from milks. Raw and pasteurized milks were found to have an appreciable proportion of curds of each type, and hence could be termed "a-b-c" milks (fig. 3). Boiled or thoroughly homogenized milks yielded curds of b and c size only ("b-c" milks) whereas human milk with its very fine curds fitted into the "c" milk class. Furthermore it was feasible to graph or chart the distribution of the a, b, and c component into what might be called an "a-b-c diagram."

This "diagram" technique proved useful in presenting the effect of

simple influences on a given sample of milk. For example, when milk was heated to 180° F. and held for various lengths of time at that temperature the plotted data showed a progressive decrease in the height of the "a" column while the height of the "c" column became steadily more elevated (fig. 4). The raw sample might be described as 80 per cent a, 14 per cent b, and 6 per cent c, whereas when the same milk had been heated at 180° F. for 60 minutes the schema became 0 per cent a, 51 per cent b, and 49 per cent c. By connecting the left corner of each rectangle representing the percentage weight of curds of a, b, and c size the typical diagram could be obtained.

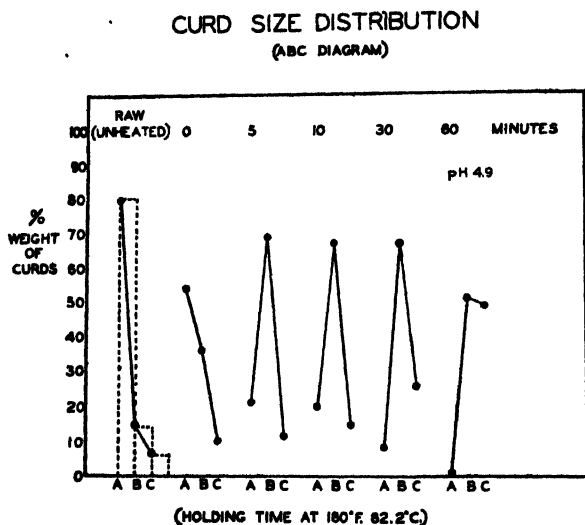


FIG. 4. Diagram showing changes which take place in curdling "a-b-c" properties as a specimen of raw milk is kept warm at 180° F. for progressive periods of time. The percentage weight of a-sized curds gradually grows smaller, and finally disappears in 60 minutes, whereas the percentage weight of c-sized curds steadily mounts until nearly 50% of the total weight of the curds is of the fine variety. (For discussion see text.)

This simple a b c system was useful with small series of specimens but proved too cumbersome when large numbers of milk samples were being codified and compared. For such circumstances it seemed essential to replace the three factor equation with a single figure, such as might be secured if the three units of the a b c code were multiplied by arbitrarily chosen factors and then added together. If the higher factors were linked to the smaller sized curds, then the smaller the average curds, the greater the "curd number."

Many different sets of incremental factors were tried out empirically in order to find the simplest which would express clearly the changes in curd size obtained when milks were modified. The simple arithmetical series 1, 2, 3 proved very satisfactory and gave dependably reproducible results. When

“a” is multiplied by 1, “b” by 2, and “c” by 3, the final total increases steadily as the percentage of small curds increases:

$$1a + 2b + 3c = \text{curd number}$$

The lower limit of this schema would be found in a milk having all the curds of “a” size:  $a=100$ ,  $b=0$ , and  $c=0$ ; curd number = 100. The maximum curd number would be obtained when all curds were “c” curds:  $a=0$ ;  $b=0$ , and  $c=100$ ; curd number = 300.

#### FACTORS AFFECTING ACCURACY OF RESULTS

*Fragility of Curds:* Freshly formed curds are friable and easily broken. In their transfer from rubber bags to jars all manipulations must be slowly and gently performed.

*Hardening:* Of the various preserving agents used in pathological laboratories, 10 per cent formalin was discovered to be the most satisfactory. It is cheap and readily available, stops instantaneously the enzymatic hydrolysis of the milk, preserves the natural shape and color of the curds, does not dissolve out the contained fat droplets, and renders the curds sufficiently firm to withstand the manipulations of sieving and washing without becoming fragmented. It does give rise to some slight shrinkage of the curds, but all sized ones are equally affected so that no disturbance in their relative proportions is produced. It precipitates the globulins from the whey in the form of delicate floccules which are washed through the No. 100 sieve and therefore do not interfere with the calculations. The greatest disadvantages of formalin lie in its odor, which makes it necessary to use a well-ventilated room for drying purposes (though not necessarily one separate from the main laboratory), and in its tendency to cause irritative dermatitis. The technician handling the curds during washing and weighing should wear rubber gloves.

*Washing:* At first (2) each sieve with its contained curds was weighed immediately following washing, and then the figure for the sieve itself when wet but empty was deducted. It was soon discovered, however, that adsorbed water was a source of appreciable error, adherent moisture being much more abundant on the fine mesh sieves with their small curds than on the coarser sieves with their larger more separated coagula. Not until the steps of transferring the curds to filter paper and permitting them to dry out were adopted could reproducible trustworthy figures for total weight be obtained. Again, the time-consuming step of filtering the wash water from the 1/100-in. sieve was eliminated because the trapped thin film of coagulated formalin-precipitated lactalbumen stippled with casein grains had a negligible weight when dried.

*Drying:* Before being washed and hardened, curds, following washing and sieving, are spread upon filter papers to dry out at room temperature. Regardless of size all curds seem to lose water at a uniform rate as illustrated

in table 1. Daily checks show a gradual loss of weight to a constant level, followed shortly by a slow steady gain. The humidity of the room air plays a role in the rate of drying, and curds weigh a little more on damp days, but no selective influence of humidity upon curds of different sizes becomes apparent. Therefore, since all comparisons are relative, and since the "curd number" calculation is based upon percentage distribution of weights, it is not necessary to wait for the drying curds to reach constant weight before weighing them. After three days the surface moisture will have evaporated, and the filter papers themselves will have become thoroughly dry. In practice the weights are taken usually after three days of drying.

TABLE 1  
*Constancy of "curd number" as related to water content of curds*

Specimen number	Date of washing	Date of weighing	Total weight of curds (a + b + c)	Calculated curd number
111A	Aug. 4	Aug. 7	20.873	280
	"	" 8	20.970	280
	"	" 9	20.898	280
	"	" 10	20.875	280
	"	" 12	20.022	280
	"	" 13	21.062	280
	"	" 14	21.041	280
	"	" 15	21.704	280
144	Aug. 13	Aug. 15	31.859	106
	"	" 16	26.943	106
	"	" 19	25.919	107
	"	" 22	25.026	107
	"	" 23	25.242	107
145	Aug. 13	Aug. 15	34.072	106
	"	" 16	28.285	107
	"	" 19	25.368	108
	"	" 22	25.422	108
	"	" 23	25.561	108
169	Aug. 13	Aug. 15	45.141	101
	"	" 16	33.713	102
	"	" 19	25.120	103
	"	" 22	24.244	103
	"	" 26	24.304	103

*Content of Pepsin:* The strength of the 0.6 per cent granulated pepsin solution recommended for the curd number test has been found to be equivalent to about 16 mgm. of rennin per ml. The milk-coagulating power of gastric juice as determined in a large group of children (12) when measured by the Helmer-Fouts method (6) has been found to range in strength between 0 and 100 mgm. of rennin per ml. Therefore, when 2 ml. of the artificial solution is added to 100 ml. of milk in the performance of the test one utilizes a standard set of circumstances which does not deviate too abnormally from intragastric conditions occurring in the young child or infant. The concentration of pepsin used, within physiologic limits, exerts but a

minor influence on "curd number," as is shown by data obtained with increasing strength of added pepsin, all other circumstances being kept constant:

Pepsin	6%	0.6%	0.06%
Curd number	243	234	229

Thus within the physiologic range, increase of pepsin concentration tends to increase the curd number slightly.

*Hydrogen-Ion Concentration:* The effective factor in the acidity of gastric juice as regards milk coagulation is the hydrogen ion. Neither the nature nor the valence of the anion is of importance. Experiments with different acids in the coagulating fluid, namely hydrochloric acid, citric acid, sulphuric acid and lactic acid, have shown the curd number to be constant for each milk so long as the pH is the same.

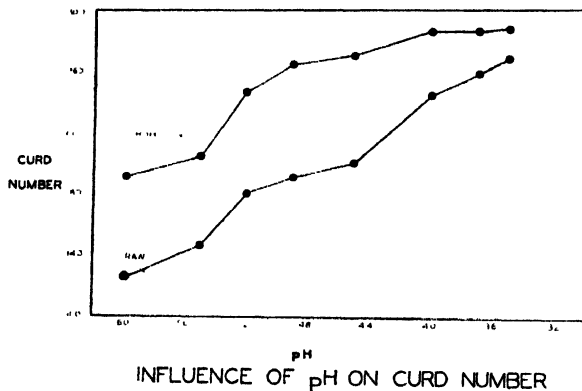


FIG. 5. Diagram showing the influence of hydrogen-ion concentration on the "curd numbers" of two specimens of milk from the same original batch. Although the curd number of the homogenized milk is consistently smaller than that of the unprocessed variety, both show a uniform rate of rise as the pH falls. Determinations cannot be safely made below pH 4.0 because the destructive proteolysis of the pepsin becomes active at this level. The unevenness of the curds is a manifestation of the range of experimental error. The homogenized milk was prepared from the raw milk.

In the first studies from this laboratory the determinations were carried out at a pH of 4.9 because of the impression that this state of acidity represented the average value for the infant's stomach. More recently obtained data (12), however, demonstrate that following ingestion of milk coagulation in the stomach takes place usually at a pH between 5.0 and 6.0, even in young adults. For all current tests on milk, therefore, pH 5.5 has been selected except for special cases.

The hydrogen ion concentration at which milk coagulates bears a direct relationship to the size of the curds which form. As the concentration grows stronger the curds become smaller. This phenomenon is illustrated in figure 5, in which the curd numbers for raw and homogenized milk from the same

source have been plotted. The curd number for the raw milk at pH 6.0 (123) was close to the minimal value obtainable (100), indicating that these curds were very large. The number for homogenized milk (190) signifies that the curds were largely of medium size. With increasing acidity the curd numbers become less widely separated as they approach the maximum figure (300).

It is not feasible to carry studies on the effect of hydrogen ion concentration below pH 4.0, since with stronger concentrations of acid the pepsin in the coagulating fluid becomes activated, and the proteolysis during the period which ensues causes significant destruction of the coagula.

*Temperature of Coagulation:* In the small range of human body temperatures for health or disease (36°–41° C.) the curdling behavior of milk showed no great changes. For the curd number technique normal body temperature (37° C.) has been selected.

*Technique of Mixing:* Immediately after adding the coagulating solution to the bag containing the milk the operator must agitate the mixture in order to obtain thorough and uniform contact between the two liquids. A second manipulation halfway through the test, at 15 minutes, proves necessary also. This must be performed in such fashion that breaking of the formed curds is avoided. In the absence of thorough mixing wide differences in curd formation were encountered among bags filled with portions of the same milk.

Three methods of manipulation of the bags for mixing purposes have been investigated: (a) gentle hand squeezing, (b) holding the ends of each bag between the fingers and moving the contents to and fro in the longitudinal axis, and (c) slipping a slender rubber tube to the bottom of the bag and blowing a stream of air bubbles from the lower end. The hand squeezing technique was found to fragment the coagula to an appreciable degree, as indicated by the high average curd number (table 2), whereas shaking produced this undesired effect less markedly, and the air bubble method least of all.

These three methods have been subjected to statistical comparison by the method of analysis of variance. On different days 15 specimens of pasteurized milk were tested by each of the three methods. With each method curd number was determined on 2 milks in duplicate, on 9 milks in triplicate, on 3 milks in 4-fold replication and on 1 milk with 6-fold replication. It is

*Analysis of variance*

Source of variation	Degrees of freedom	<sup>a</sup> (squeezing)		<sup>b</sup> (shaking)		<sup>c</sup> (bubbling)		F 5%	1%
		Mean square	F ratio	Mean square	F ratio	Mean square	F ratio		
Between milks ...	14	637.4	8.45	964.0	47.6	21.50	1.39	2.00	2.66
Within milks (error) .....	34	75.4	..	20.3	..	15.47	.....	.....	.....

evident that variation from milk to milk was small in method (c) and not significantly greater than the error. Method (b) showed the smallest error and is clearly superior to method (a). The variance of single curd number measurements with method (b) increases with the curd number and may be estimated as  $\sigma = 0.76$  (curd number - 100). In the light of these considerations, and because the air-bubbling technique was time-consuming and cumbersome, method (b), that of gentle hand shaking, was selected as the recommended procedure for the mixing maneuver.

TABLE 2

*Comparison of pasteurized milks processed by (a) squeezing, (b) hand shaking and (c) air-bubble technique at pH of 5.5*

Date	No. of exps.	a		b		c	
		Curd Nos.	Aver. curd Nos.	Curd Nos.	Aver. curd Nos.	Curd Nos.	Aver. curd Nos.
5/1	6	120-120-124 132-140-141	130	103-104-104 104-106-108	105	113-114-115 116-118-121	116
5/2	2	106-108	107	104-107	105	106-108	107
5/5	2	131-136	133	117-120	118	103-105	104
5/9	3	155-167-173	165	130-136-141	135	122-125-131	126
5/15	3	116-118-134	123	118-123-128	123	114-116-123	118
5/27	3	130-133-146	136	117-122-122	120	127-135-136	133
5/29	3	132-144-152	137	108-109-112	109	119-119-127	122
5/30	3	130-144-152	142	147-153-159	153	115-117-118	117
5/2	4	134-140-144 147	141	127-128-128 136	130	108-111-112 116	112
5/3	4	131-134-143 151	139	120-120-122 128	122	115-116-124 124	120
5/5	3	119-121-137	126	112-115-124	117	104-105-106	105
5/9	4	136-142-144 158	145	155-155-163 169	161	121-121-126 130	125
5/10	3	140-159-164	154	137-138-148	141	112-119-122	117
5/24	3	156-164-171	164	122-124-132	126	118-120-126	121
5/25	3	129-132-134	132	108-111-113	110	102-103-105	103
Average			138.3		125.1		116.4

TABLE 3

*Variance of curd number (shaking method)*

	Curd number		
	100-120	120-140	140-180
Single measurements . . .	$\pm 6$	$\pm 10$	$\pm 13$
Duplicate measurements . . .	$\pm 4$	$\pm 7$	$\pm 10$
Triplicate measurements . . .	$\pm 3$	$\pm 6$	$\pm 8$

*Exactness of Results (Shaking Method):* Table 3 shows the accuracy of measurement for test specimens of pasteurized milk, taking 5 per cent



fiducial limits. Since pasteurized milk rarely gives a curd number higher than 180, homogenized milk, which possesses a spread centering between 200 and 280, was taken to explore the exactness of the curd number method in the range between 180 and the maximum 300. Analysis of the data in a series of tests taking 5 per cent fiducial limits of curd number 200-275 showed that the accuracy for single measurement was 8.4, duplicate measurement 6.0, and triplicate measurement 4.9. There was no significant trend of variance as the mean increases from 205 to 272. That this calculated variation for homogenized milk is less than that for pasteurized milk indicates that the accuracy of the curd number test is contingent in part upon the type of milk processed.

#### FAT CONTENT OF THE CURDS

In order to have a clearer understanding of the nature of these artificially produced milk curds their butter fat content was compared with the values for the fluid milk before coagulation. Curds after being thoroughly dried out at room temperature were extracted with ether in a soxhlet apparatus and the percentage of ether soluble substance calculated as butter fat. Table 4 shows the results obtained with raw, pasteurized and homogenized milks. Generally speaking, about 90 per cent of the butter fat was caught in the curds, irrespective of the original form of the milks. (Check deter-

TABLE 4  
*Fat content of curds*

Specimen number	Type of milk	Original fat content of the milk (gm. per 100 cc.)	Weight of curd produced, by 100 cc. milk	Fat content in curds from 100 cc. milk	Total fat caught in curds	Fat per 100 gm. of air-dried curd	Residue per 100 gm. of air-dried curd
		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>%</i>	<i>gm.</i>	<i>gm.</i>
1	Raw	4.10	7.22	3.72	90.6	51.5	48.5
2	Raw	4.12	7.31	3.68	89.5	50.3	49.7
3	Raw	3.88	7.73	3.50	90.2	45.1	54.0
4	Past.	3.92	6.91	3.41	86.9	49.3	50.7
5	Past.	3.92	6.84	3.61	92.0	52.1	47.9
6	Homog.	4.21	7.32	3.90	92.6	53.3	46.7
7	Homog.	4.22	7.37	3.89	92.5	52.8	47.2
8	Homog.	4.30	7.29	3.91	90.9	53.6	46.4
9	Homog.	4.30	7.15	3.91	90.9	54.7	45.3
10	Homog.	4.30	7.17	3.93	91.4	54.8	45.2
11	Homog.	4.30	7.11	3.93	91.4	54.9	45.1

minations demonstrated the balance to be contained in the whey. Interestingly, the analyses bring out that with market milks containing about 4 per cent butterfat the curds which form are half protein and half fat, and that when the fat content of the milk is lower or higher the fat content of the curds fluctuates correspondingly. These observations corroborate the find-

ings of Freudenberg (5), and coincide with the experience of cheese-making practice. If the curds which form within the human stomach are similar in composition to those produced *in vitro*—and there is no evidence to suggest they are not—then one would not expect to find milks high in butter fat appreciably retarding the emptying time of the stomach, since the increased fat being trapped in the casein coagula is not free to exert its specific stimulus upon the gastric mucosa.

#### PRACTICAL APPLICATIONS

The secretion of acid and pepsin by the gastric mucosa following the taking of milk varies from individual to individual and is inconstant even in the same subject on successive determinations, being dependent upon such

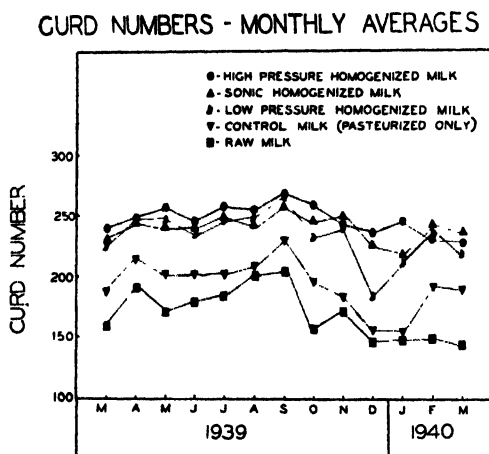


FIG. 6. Average "curd numbers" of milks used in a clinical feeding study. A seasonal swing is present in all the milks, the numbers being lowest in March, climbing to a wavelike crest in September, and receding to another low in the following Spring. The rise in values for pasteurized milk during February and March, 1940, results from the change to a high pasteurizing temperature—160° F. for 30 minutes instead of the 145° F. previously used (from table 5). The curds were prepared for measurement at pH 4.9 with the hand-squeezing technique.

influences as age, state of health and nutrition, emotional balance, innate capacity and presence or absence of fatigue (8, 12). This changeful composition of the gastric juice would be expected to reflect itself in altered patterns of coagulation even when the character of the milk itself is kept constant. With the curd number test each component in the curdling system can be individually altered and its significance evaluated in terms of physiologic values. Such extrinsic factors as the effect of hydrogen ion concentration changes, the alterations in relative content of pepsin or hydrochloric acid or both in the coagulating fluid, the rapidity with which coagulating solution is added, the significance if any of the temperature of the

swallowed milk and the prominence to be attached to the motor activity within the moving system all become subject to analysis by means of this new approach.

For the past two years the curd number test in its present form has been subjected to daily use and scrutiny. It has been found adaptable for the study of differences among raw milks, changes produced by various processes and modifications upon these same milks and the influence of factors present in the stomach upon curd formation. Thus raw milk specimens from diverse sources of supply and at the various seasons have been found to present great differences among themselves as reflected by curd number determinations. Processing and changes made in the milk become similarly reflected (fig. 6). The influence of increments of added acid upon curd size is indicated by progressive elevations in the curd numbers (fig. 5). Breakdown of fat particles by homogenizing results in a striking increase in the curd number values (fig. 5). Similarly in the heating of milk progressive increase of either the temperature or the time factor produces an elevation in the values obtained (figs. 7, 8). A detailed report of the observations gained by the use of the curd number test in the evaluation of current practices of processing cow's milk for marketing purposes and of modifying milk for infant feeding will be published separately (14).

#### CURD NUMBER AND CURD TENSION

The curd tension test, though limited by its inflexible rigidity to use with undiluted milk only (9), is simpler, quicker and more economical than the curd number test, and gives results more promptly. It is specially suited for work in the dairy plant and testing laboratory, where it can be used for checking on the day-to-day output of milk for soft or hard curd properties. The curd number test on the other hand is more sensitive, broader in scope and offers richer potentialities for research investigations on milk and milk products. The two methods can be considered to complement one another; each may be used to check the other.

A great mass of comparative data bearing on the relationships between these two tests has been accumulated (14). Generally speaking, the results obtained by the two methods run more or less parallel, indicating that the techniques measure similar or closely related properties of milk. For example, the readings for curd number and for curd tension on milks held at constant temperatures for varying periods of time have shown close correlation in their rates of changes (figs. 7, 8). Milks from different sources, however, often possess similar curd numbers but differing curd tension values, and vice versa. On several occasions certain manipulations of milk have resulted in marked changes of curd number in the direction of soft curd character without appreciably altering the curd tension values.

## CURD NUMBER AND DIGESTIBILITY

The problem of immediate importance is to determine the validity of the "curd number" test as an index of digestibility. Does this arbitrarily established procedure measure some significant property of milk which is responsible for differences in digestibility? Does a low curd number actually

## INFLUENCE OF 180°F (82.2°C)

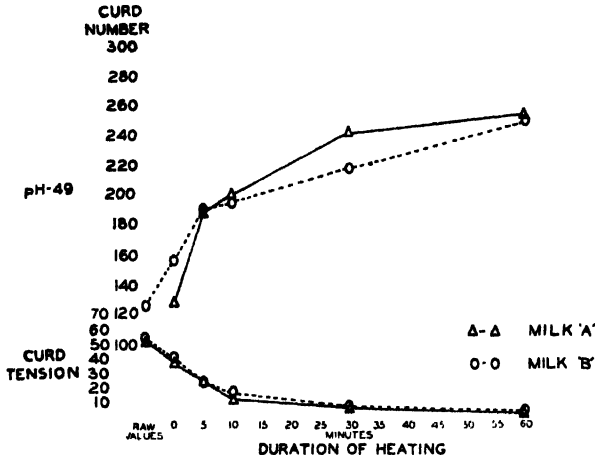


FIG. 7. Comparison between curd number and curd tension for milk heated to 180° F. (82.2° C.) and held for various minutes.

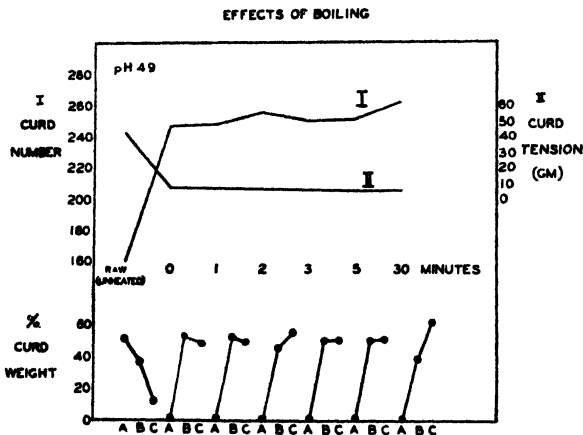


FIG. 8. Curd tension and curd number for milk heated to boiling temperature and held for various minutes—correspondence with a, b, c diagram demonstrated.

correspond to "hard-curd milk," and does milk with a high number prove to be "soft-curd" and more readily digestible? If so, where does the dividing-line or threshold fall?

In order to obtain pertinent information concerning the above questions, determinations of curd number were secured at frequent regular intervals

during the course of a 15-month infant-feeding study (13) in which it was found that milks processed by (a) sonic homogenization, (b) "low pressure" homogenization and (c) "high pressure" homogenization, and used as the basic food for infants, were comparable in ease of digestibility with pasteurized milk boiled for five minutes. More than 200 normal infants were successfully raised on each type of milk without exhibiting symptoms or signs of digestive disturbances attributable to imperfect utilization of the milk within the gastro-intestinal tract. Figure 6 summarizes the "curd number" data for the milks obtained from some 400 tests, as measured at pH 4.9 with the "hand squeezing" technique. The raw milk gave the lowest value; the pasteurized milk fell next in line, whereas the three varieties of homogenized milk, and boiled milk, were grouped together at a higher level. Although a great seasonal swing was reflected in all the milks, no concomitant changes in the infant's feeding behavior became manifest. Save for an occasional transient exception, the majority of homogenized milk specimens from the experimental study gave curd numbers which when adjusted by calculation to the circumstances of coagulation at pH 5.5 with mixing by shaking (test conditions which seem most satisfactory) fell at or usually above 200. That these three varieties of homogenized milk processed by three different and distinct techniques were equally and efficiently metabolized by such a large group of healthy normal infants seems evidence enough to characterize them all as soft curd milks as this designation is applied (3, 4). One may generalize further and point out that other homogenized milks having curd numbers at or above 200 would presumably be tolerated with comparable ease of digestibility, provided of course that their sanitary and other features were not unsatisfactory. Whether other forms of milk not homogenized can be subjected to the same criterion remains to be determined, as is also the problem of evaluating the digestibility of less thoroughly processed homogenized milks which yield curd numbers below this observed limiting value.

#### SUMMARY AND CONCLUSIONS

When a specimen of milk is made to coagulate within an artificial curdling device under rigidly controlled conditions, the curds which form manifest a size distribution which appears to be a constant physical characteristic of the milk undergoing test. After hardening, drying, sieving and weighing the masses of curds thus obtained and then applying to the weight data a so-called "a-b-c" formula empirically derived, it is possible to arrive at a "curd number" which epitomizes the milk's curdling qualities. This proposed technique for curd number has been subjected to critical analysis and found to be experimentally useful in problems dealing with the coagulating properties of cow's milk preparations as related to human digestion. Curd

number has been found in general to run parallel to curd tension, though broader in scope and with greater applicability to research.

#### ACKNOWLEDGMENT

We are grateful to Dr. J. H. Austin, Department of Research Medicine, University of Pennsylvania, for assistance in the statistical analyses of the data. Appreciation is due also to Dr. Leslie Chambers for many critical suggestions, to Mr. A. P. Hands and Miss Gladys Rosenstein for technical assistance, and to the Submarine Signal Co., Boston, Massachusetts, for construction of the apparatus.

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# CURD STRENGTH OF EVAPORATED MILK\*

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In the milk and cheese industry the subject of curd strength as expressed in grams is commonplace. Limited information, however, relating to this subject is available for evaporated milk. A study was undertaken to obtain information of this and closely allied subjects.

## PROCEDURE AND RESULTS

A desired range of samples was procured through the cooperation of the Evaporated Milk Association. Single plant samples from 19 states, samples from two plants in Ohio and also Pennsylvania and from 3 plants in Wisconsin were obtained. These samples were from batches made between June 10 and 15, 1940. Composition, sterilization data and place of manufacture appear in table 1. These values supplied by the Association were studied statistically before our studies were undertaken.

To obtain curd tension values both concentrated and diluted samples were used. Curdling periods were extended whenever the Hill, Miller or Geneva (2, 3, 1) procedures gave negative results. A range of acid percentage was also studied for the Miller procedure.

The creaming capability of re-constituted samples was also studied. Fat percentages in various layers of 1000 ml. amounts were used to study the creaming properties. All samples were set at 4.5° C. (40° F.) for 18 hours. In one series the fat percentages were determined in the upper 100 and lower 900 ml. In the other gravitations the fat percentage was multiplied by 4.1, the normal mean percentage factor for creaming power of raw milk, and this percentage value divided by two gave the amounts of the 1000 ml. to be taken for the upper and center layers. The remainder was used as the lower layer. All samples were observed for flavor, body and color.

A review of the analyses reveals that the mean fat percentage of the 26 samples is 7.94. Twenty-three samples fall within the fat error tolerance of the mean value, the other three samples within 0.1 per cent deviation from the mean. This degree of uniformity of composition has been achieved by a system of technical control, inspection and checking by the industry which has significance in this study since it enabled the supplying of 26 comparable samples as far as fat content is concerned.

The degree of uniformity of the total solids is quite comparable to that of the fat content. The mean value for total solids percentage is 26.23.

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The median, 26.15, deviates less than 0.1 per cent from the mean value. The mean value within the inter-quartile range, 26.19, falls mid-way between the median and mean values. More significant, however, is the fact that with one exception all total solid values vary less than 0.5 per cent. This means that the samples are quite comparable for total solids percentages. This is on the premise that total solids are more difficult to control than fat

TABLE 1

*Composition, sterilization data, place and date of manufacture of the evaporated milk samples used*

Sample	Date of mfr.	State	Milk fat	Total solids	Sterilization	
					Time	Temp.
			<i>Per cent</i>	<i>Per cent</i>	<i>Min.</i>	<i>°F.</i>
1	6/10/41	Pennsylvania	7.93	26.13	16	244
2	6/12/41	New York	7.92	26.18	16	242
3	6/11/41	Oregon	7.93	26.11	15	243
4	6/10/41	Ohio	7.96	26.08	14	242
5	6/10/41	Virginia	7.93	26.30	15	243
6	6/14/41	Washington	7.95	26.02	15	243
7	6/12/41	Wisconsin	7.93	26.06	15	243
8	6/12/41	Texas	8.01	26.05	14	242
9	6/11/41	Iowa	7.93	26.37	15	242
10	6/13/41	Minnesota	7.94	26.05	15	242
11	6/10/41	Colorado	7.93	26.05	14	242
12	6/14/41	Pennsylvania	7.95	26.11	15	246
13	6/14/41	Illinois	7.93	26.15	15	242
14	6/15/41	California	7.85	26.01	14.5	244.5
15	6/11/41	Ohio	7.94	26.95	13.5	245.5
16	6/13/41	Alabama	7.96	26.25	15	242
17	6/10/41	Kentucky	7.95	26.31	15	244.5
18	6/11/41	Mississippi	7.94	26.42	14	242
19	6/10/41	Maryland	7.95	26.43	15	242
20	6/11/41	Tennessee	7.97	26.28	15	245
21	6/15/41	Michigan	7.94	26.16	15	245.5
22	6/15/41	Wisconsin	7.94	26.30	15	241.5
23	6/12/41	Idaho	7.94	26.40	11	244
24	6/12/41	Utah	7.95	26.51	13.5	243
25	6/11/41	Wisconsin	7.95	26.26	15	243.5
26	6/11/41	Indiana	7.95	26.07	15	240

percentages. Therefore these samples represent the maximum in likeness achievable in total solids control.

The values in table 2 give the curd strength of the complete series by the Hill, Miller and Geneva methods as described by Dahlberg *et al.* (1). This investigation was in progress when the American Dairy Science Association Procedure for Measuring Curd Strength was published. Present investigations are being conducted with the approved procedure of the Association adopted in September, 1941. The diluted samples were equal parts by weight of evaporated milk and water. The values reveal that all the undiluted samples fall within the soft curd milk range as defined by Hill (2). Over two-thirds of the undiluted samples meet the requirements in Hill

units as set forth by the American Medical Association for normal homogenized milk. All undiluted sample curd strengths meet the American Medical Association Standards when expressed in Miller Units (3).

TABLE 2  
*A presentation of the curd strength of evaporated milk*

Sample	Undiluted			Diluted*		
	Hill	Miller	Geneva	Hill	Miller	Geneva
1	20	10	0	4	2	0
2	19	10	0	4	2	0
3	20	12	0	4	2	0
4	21	10	0	5	2	0
5	30	12	0	4	0	0
6	30	12	0	6	0	0
7	22	8	0	3	0	0
8	30	12	0	6	2	0
9	20	8	0	4	2	0
10	20	10	0	4	4	0
11	16	12	0	4	3	0
12	16	12	0	4	4	0
13	12	12	0	4	3	0
14	30	10	0	4	4	0
15	26	10	0	4	6	0
16	22	12	0	4	2	0
17	22	14	0	4	4	0
18	18	12	0	4	4	0
19	22	0	0	4	0	0
20	22	0	0	4	0	0
21	16	0	0	4	0	0
22	16	0	0	4	0	0
23	18	0	0	6	0	0
24	20	0	0	3	0	0
25	26	0	0	4		0
26	24	0	0	5		0

\* Diluted for this series refers to equal parts by weight of evaporated milk and water.

The diluted values present samples with a very low curd tension, 6 or below expressed in Hill or Miller units. Homogenized milk as sold seldom has a curd value as low; most samples having values within a 12 to 20 range. This is well established and verified by unpublished data in our files. The fact that curd values were not attainable due to lack of setting with the Geneva procedure stimulated further investigations with this procedure.

The flavor, body, and color comments are not included in table 2 as all samples rate a "good" on flavor and body. Eighteen samples were comparable in color with 4 being slightly lighter and 4 slightly darker than the 18. These variations were not significant.

Four samples which failed to set curds in 10 minutes with the Miller and Geneva solutions were tested after 30 and 60 minute intervals, and failed to give curds within these periods. Both solutions set curds with the four selected samples after 24 hours. The Miller values were 15, 12, 5 and 4;

whereas under the same condition the Geneva values were 12, 12, 12 and 6. In the above trials a 0.4 per cent HCl solution was used for the Miller solution. Increasing this concentration 0.1 or 0.2 per cent gave quite normal results with the samples which failed to set with the standard Miller solution. Likewise samples which set normally with a 0.4 per cent HCl Miller solution failed to set when the concentration was decreased 0.1 or 0.2 per cent. -Slight variations in reaction may give decided difference in values when measurements are made after 10 minutes.

To answer the question concerning the changing of curd strength of evaporated milk after being held at room temperature for 6 months, the curd tension on all samples was repeated during December. The values obtained are not presented as the results showed no change in curd strength as measured by the Hill, Miller or Geneva procedures as outlined in this study which could be attributed to aging for 6 months at room temperature.

The creaming tendency of re-constituted evaporated milk is important. Extensive creaming studies were made with 7 samples. In the first series the re-constituted evaporated milk was creamed in 1000 ml. cylinders for 18 hours at 4.5° C. (40° F.). The upper 100 and lower 900 ml. were analyzed. In the second series the fat percentage multiplied by 4.1 and divided by 2 as previously described gave the amounts of upper and center portions, the remainder being regarded as the lower layer.

TABLE 3

*Creaming tendencies of re-constituted evaporated milk as measured by two procedures*

Sample	Per cent fat dil.	Procedure 1*		Procedure 2†		
		Per cent fat 100 ml. upper	Per cent fat 900 ml. lower	Upper	Center	Lower
1	3.7	3.7	3.7	3.8	3.7	3.7
2	4.0	4.0	4.0	4.0	4.0	4.0
3	3.7	3.7	3.6	3.7	3.7	3.6
4	3.9	3.9	3.9	3.9	3.9	3.7
5	3.7	3.7	3.6	3.8	3.7	3.7
6	3.8	3.8	3.8	3.8	3.8	3.8
7	3.9	3.9	3.7	3.9	3.8	3.7

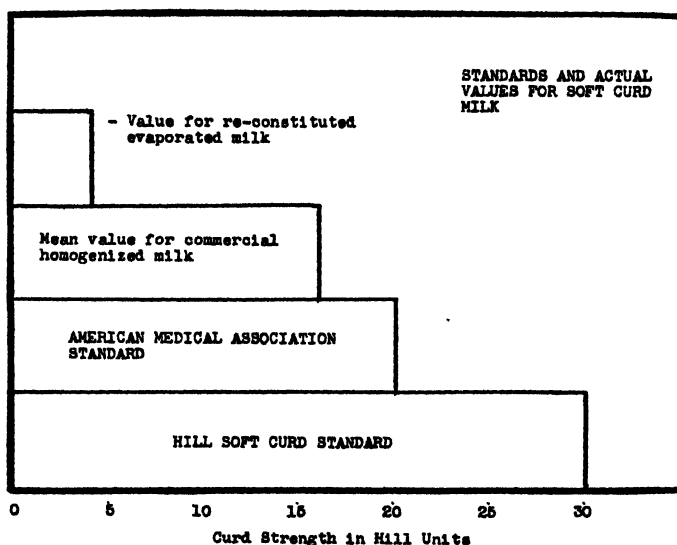
\* These layers were obtained by removing 100 ml. from a quart of milk held at 40° F. for 18 hours.

† These layers were obtained from 1000 ml. of milk set at 40° F. for 18 hours. The upper and center layers were obtained by multiplying the fat percentage by 4.1, the creaming factor for normal milk, and dividing the result equally for the two layers. The remainder was taken as the lower layer.

The creaming tendencies are summarized in table 3. It is apparent that as far as practice is concerned the evaporated milk samples have their creaming tendency eliminated. The variations in fat percentages in table 3 are greater than those recorded in table 1. The values in table 3 were obtained with a modified Babcock procedure whereas those in table 1 were

ether extract analyses. A variation in fat content of not more than 0.2 per cent was found between the various layers when compared to the re-constituted control.

Further experimentation with 6 samples revealed that dilution as great as 5 per cent of milk and 95 per cent of water failed to induce creaming properties. The samples were selected at random; the percentages of evaporated milk used were 5, 25, 50, 75, and 95. After creaming at 4.5° C. (40° F.) for 18 hours lower and upper layer means checked within 0.2 of the fat percentage of the mixed samples by the Babcock procedure. For example, all samples when mixed with equal amounts of water by weight gave a mixture containing 4.0 per cent of fat. The upper layer had a mean value of 4.0 per cent; which was also the percentage present in the lower layer; cor-



Curd strength of re-constituted evaporated and homogenized milk compared to the American Medical Association and Hill Standards.

responding values for the 75 per cent evaporated milk and 25 per cent water mixtures were within 0.1 of a value of 6 per cent. The mixture with 25 per cent of evaporated milk gave values within the 0.1 range of 2.0 per cent. The 5 per cent mixtures contained 0.5 per cent fat and the lower and upper layers checked within 0.2 per cent of this value. The mean values for the mixture, upper and lower layers for the 95 per cent evaporated milk mixture were 7.4, 7.5, and 7.4. These results established that dilution percentage is not a factor in the non-creaming of diluted evaporated milk.

#### CONCLUSIONS

The curd strength of re-constituted evaporated milk is far less than that of commercial homogenized milk. Its curd strength is well within the standard set by the American Medical Association of 20, measured in Hill units.

Re-constituted evaporated milk, when mixed with equal volumes of water by weight, will not lose its homogeneous properties when held at 4.5° C. for 18 hours.

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# VITAMIN A AND CAROTENE REQUIREMENTS FOR THE MAINTENANCE OF ADEQUATE BLOOD PLASMA VITAMIN A IN THE DAIRY CALF<sup>1</sup>

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A number of studies have been reported on the carotene requirement of dairy cattle without complete agreement. The carotene requirements as determined by Guilbert and coworkers (7, 8) have been supported by Ward *et al.* (19), Moore (17), and Halverson *et al.* (9). Reproduction apparently increases the requirement (4, 6, 7). However, from studies reported by Meigs and Converse (16) and Phillips *et al.* (18) it appears that the growing calf requires more carotene than the minimum requirement proposed by Guilbert *et al.* (7, 8).

In a recent study of the relationship of avitaminosis A to ascorbic acid in the young bovine (3) it was found that the blood plasma ascorbic acid was contingent upon the blood level of vitamin A, in particular when plasma vitamin A values fell below 10 $\gamma$  per 100 cc. If blood plasma levels of 5–7 $\gamma$  per 100 cc. or less continued, vitamin A deficiency symptoms and pathology appeared. These data suggested that the blood plasma vitamin A was an index of the status of the vitamin A nutrition of the calf. On the basis of these preliminary results it seemed possible that the minimum requirements of carotene and vitamin A could be determined by using the blood plasma vitamin A as a critical measure of vitamin A nutrition. The results of such an experiment are herewith reported.

## EXPERIMENTAL

The data given in this paper were secured from 6 calves (3 Guernseys, 2 Holsteins, and a Holstein-Brown Swiss) placed on experiment when they were approximately 4 weeks of age. They were divided into three lots of one Holstein and one Guernsey each so that three ingestion levels of vitamin A or carotene could be simultaneously compared. The calves were fed the low carotene ration of Walker (18) which was composed of white corn 22 parts, linseed oil meal 23 parts, wheat middlings 11.5 parts, oat mill feed 40 parts, ground limestone 3 parts, iodized salt 0.5 parts and irradiated yeast 0.1 part. The ration averaged about 5–8 $\gamma$  carotene per pound. Skimmed milk supplements were fed during the early weeks of the experiment.

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Blood plasma carotene and vitamin A determinations were made at weekly intervals throughout the experiment by an adaptation of Kimble's method (14). Results of the vitamin A analyses were expressed in terms of micrograms on the basis of measurements with pure vitamin A.<sup>2</sup> Occasional ophthalmoscopic examinations were made for the purpose of correlating with the blood analyses. Body weights were taken at weekly intervals and all animals were closely watched for gross symptoms of vitamin A deficiency.

Supplements of crystalline carotene in cottonseed oil, shark liver oil, and alfalfa were fed as source materials for vitamin A or carotene. The solution of carotene in cottonseed oil was prepared by dissolving one-half gram of carotene in a few cc. of chloroform and adding this to 600 cc. of cottonseed oil previously heated in an oven to 70–80° C. The resulting solution was cooled immediately under running tap water and stored in the refrigerator until used. Each solution was analyzed for its exact carotene content before use. The shark liver oil was an excellent quality, high potency, straw-colored oil.<sup>3</sup> The potency of the oil was checked by means of the antimony trichloride reaction (5).<sup>2</sup> The alfalfa hay was Nebraska-grown hay of good quality and color. The carotene content of the hay was determined by the method of Hegsted *et al.* (11).

It was planned to determine the blood plasma vitamin A levels which would be adequate, borderline, and inadequate for the calf, and the required ingestion of vitamin A necessary to support these blood levels. Further, it was planned to determine what were the carotene requirements for the maintenance of an adequate blood plasma vitamin A.

After the calves were placed on experiment it required 30 days to deplete their stores and to reduce the blood plasma vitamin A to a low level, and thus properly condition them for the determination of necessary vitamin A intakes and plasma levels. During the next 90 days an attempt was made to stabilize the blood plasma vitamin A levels at 10–12 $\gamma$  per 100 cc., 8 $\gamma$  per 100 cc. and 4 $\gamma$  per 100 cc. in groups I, II, and III, respectively. These were levels which previous results indicated would be adequate, borderline, and inadequate for vitamin A nutrition. On the basis of the results obtained vitamin A was fed at constant levels for the next 90 days. The ingestion was based upon body weight corrected weekly. Following this period, supplements were withdrawn to ascertain if any storage of A had occurred and to properly stabilize the calves for determining the carotene requirements. The depletion time required for this was 35 days. Three levels of carotene (20, 40 and 60 $\gamma$  per kg. of body weight per day) were then fed for 75 days. Since it became apparent that these levels were inadequate, the carotene in-

<sup>2</sup> The results of the vitamin A analyses may be subject to slight modification as the shark liver oil or the blood plasma might possibly contain chromogens other than vitamin A with respect to the antimony trichloride reaction.

<sup>3</sup> Generously supplied for these experiments by Bioproducts Inc., Astoria, Oregon.

TABLE 1

*The relation of blood plasma vitamin A to carotene and vitamin ingestion and to growth*

[illegible]

\* In the period 30-120 days the intakes were experimentally varied and thus the blood levels fluctuated. The figures given are averages and are somewhat higher than the results obtained later on constant intakes. The figures for the remitting periods represent the values obtained on the respective intakes after the blood levels became relatively constant.



take for all calves was increased to 100 $\gamma$  per kg. of body weight for 15 days after which the intakes were modified as needed and fed for another 35 days. Thus the experiment extended over a 370-day period.

#### RESULTS

The data in table 1 show the effect of blood plasma vitamin A on growth. When the blood plasma vitamin A values were 10 $\gamma$  or above per 100 cc. the calves gained a pound or more of weight per day. Lower concentrations of blood plasma vitamin A were accompanied by a decreased rate of growth and practically complete inhibition of growth followed when blood plasma vitamin A fell to levels of 4 $\gamma$  per 100 cc. or less. A noticeable lag was evident between the blood plasma vitamin A and the growth response. When the blood plasma vitamin A was sharply reduced decreased growth did not result for as long as 30 days afterward, thus indicating that the blood plasma vitamin A provided a much more sensitive criterion of the status of vitamin A nutrition than growth. The growth rate was good on intakes of 18 $\gamma$  of vitamin A per kg. of body weight per day, but was markedly inhibited on the low intakes of 6 $\gamma$  per kg. of body weight.

As shown in table 1, plasma vitamin A levels of 10–12 $\gamma$  per 100 cc. prevented the appearance of any deficiency symptoms over a 6 month period (Calves 1 and 2). Levels of 7–8 $\gamma$  per 100 cc. were borderline in the prevention of the development of vitamin A deficiency symptoms (Calves 3 and 4). If blood plasma vitamin A levels of 4–6 $\gamma$  per 100 cc. or less were allowed to persist, deficiency symptoms invariably developed (Calves 5 and 6).

The vitamin A intakes necessary to maintain the different levels of blood plasma vitamin A were consistent for all calves except number 4. In general, the ingestion of 18, 12, and 6 $\gamma$  of vitamin A per kg. of body weight per day resulted in adequate, borderline and inadequate blood plasma concentrations of vitamin A.

With these values established the vitamin A supplements were withdrawn on the 210th day for a period of 35 days. The rapid fall in plasma vitamin A and the appearance of symptoms indicated that even on the highest level of ingestion storage of vitamin A was not great. At the end of this period a small amount of vitamin A was given calves 5 and 6 to raise their blood plasma vitamin A to a par with the other calves. All calves were then placed on carotene supplements as the source of vitamin A. The calves in lots I and II were given the carotene solution and those in lot III were given alfalfa hay as a source of carotene. Carotene was first fed at intakes of 60, 40 and 20 $\gamma$  per kg. per day. The 20 and 40 $\gamma$  levels were totally inadequate and even the 60 $\gamma$  level was definitely subminimal for maintenance of adequate blood plasma vitamin A and the prevention of deficiency symptoms. During this period the blood plasma carotene content increased to double the minimum carotene level suggested by Moore (17). These results are in

accord with earlier unpublished data obtained by us in which it was found that vitamin A deficiency in calves developed on carotene intakes of 50–60 $\gamma$  per kg. per day.

On the 320th day the carotene ingestion level was increased to 100 $\gamma$  per kg. for all calves. Within a short time it became evident that this level was more than adequate for the Holsteins and inadequate for the Guernseys, indicating that there was a breed difference in the carotene requirements. The carotene intakes were then adjusted to 75 $\gamma$  per kg. of body weight for the Holsteins and 125 $\gamma$  for the Guernseys, and the Holstein-Brown Swiss maintained at 100 $\gamma$  per kg. These intakes of carotene resulted in blood plasma vitamin A values of 8–10 $\gamma$  per 100 cc. over a 35-day period. The results of these experiments indicated that no difference existed in the availability of the carotene in cottonseed oil and the carotene as present in alfalfa. Whether the ingestion of an equivalent carotene level from alfalfa would in time prove superior to crystalline carotene dissolved in oil was not answered by this study.

The data covering the relation of carotene and vitamin A are summarized in table 2. It is seen that an intake of 18 $\gamma$  of vitamin A per kg. of body

TABLE 2

*Summary data on the relationship of carotene and vitamin A intake to the blood level of carotene and vitamin A*

Daily intake		Blood plasma vitamin A		Blood plasma carotene	
Carotene	Vitamin A	Holstein	Guernsey	Holstein	Guernsey
$\gamma$ /kg.	$\gamma$ /kg.	$\gamma$ /100 cc.	$\gamma$ /100 cc.	$\gamma$ /100 cc.	$\gamma$ /100 cc.
No supplementation					
5–10*	0	3	3	11	19
Vitamin A supplementation					
5–10*	6	6	5	14	15
5–10	12	8		13	
5–10	18	12	12	11	22
Carotene supplementation					
25–35	0		4		28
35–45	0	5		8	
45–55	0	5		12	
55–65	0	6	4	16	71
65–75	0	7		32	
75–85	0	9		58	
95–105	0		7		105
125–135	0		10		137

\* Supplied by basal ration.

weight was required to maintain a blood plasma level of 10–12 $\gamma$  per 100 cc. for both Guernseys and Holsteins. This requirement is an addition to the residual carotene supplied by the basal ration. The response to graded

doses of carotene showed that intakes of 75–85 $\gamma$  per kg. of body weight per day were necessary to bring the blood vitamin A to 10 $\gamma$  per 100 cc. in the Holstein. To reach the same level in the Guernsey 125–135 $\gamma$  per kg. was needed. Thus on the basis of blood analyses vitamin A was found to be 5 to 8 times as efficient as carotene in maintaining an adequate blood plasma vitamin A. These values are in good agreement with the ratios proposed by Guilbert *et al.* (7, 8).

When carotene was fed as the sole source of vitamin A, the Holsteins required 50–70 $\gamma$  of carotene per 100 cc. of blood plasma for the maintenance of a vitamin A level of 10 $\gamma$  per 100 cc. The requirements for the Guernsey were definitely higher. They required from 110–140 $\gamma$  of carotene per 100 cc. to maintain an adequate plasma vitamin A level.

#### DISCUSSION

The carotene and vitamin A requirements found in these experiments are not without support from other investigations. Meigs and Converse (16) found that for young calves vitamin A intakes up to approximately 22 $\gamma$  per kg. and carotene intakes up to approximately 87 $\gamma$  per kg. of body weight per day were inadequate. They interpret their data as indicating a higher need for the first 3–4 months of life. Our experiments extended over more than a year. Booher *et al.* (2), Jeghers (13) and others have found that the human adult requires somewhat higher absolute levels of vitamin A or carotene than the minimum requirements postulated by Guilbert *et al.*

The minimum values indicated by our work are about equal to those shown to be necessary for normal reproduction and optimal dark adaptation by Guilbert *et al.* (7, 8), but they are 2–3 times as great as their minimum intakes. It may be that intakes which prevent nyctalopia under conditions of their experiments do not represent true physiological minima. In this regard Moore (17) showed that on a carotene intake of approximately 35 $\gamma$  per kg. nyctalopia may be prevented and the outward appearance may be normal, but it may not be sufficient to keep the cerebrospinal pressure normal as indicated by papillary changes. In Moore's experiments (17) papillary edema appeared before nyctalopia in several calves, and carotene intakes of 35 $\gamma$  per kg. did not cure papilledema over a year's period in some calves.

In studies on human nutrition several investigators (10, 12, 15) have shown that poor dark adaptation may occur before vitamin A deficiency becomes so extreme as to result in clinically manifest symptoms such as night blindness and xerophthalmia. Bodansky *et al.* (1) have concluded that the plasma vitamin A is a considerably more sensitive indicator of vitamin A deficiency than is dark adaptation. They found that in infants with a low concentration of blood plasma vitamin A normal dark adaptation could be restored without increasing the level of plasma vitamin A.

Guilbert and coworkers (7) have demonstrated normal or nearly normal

growth over extended periods on their minimum carotene intakes. In our studies we have found that gains in weight may be expected for some time after sub-minimal plasma vitamin A values have been reached, and that some weight gain may continue for long periods on carotene intakes low enough to allow a marked deficiency to develop.

Moore (17) has reported that when plasma carotene values fall to 13 $\gamma$  per 100 cc. or lower in Holstein calves a vitamin A deficiency is likely to develop. In our experiments the carotene values have often decreased to this level before outward deficiency symptoms appeared. However, it was necessary to maintain the plasma carotene values considerably above 13 $\gamma$  per 100 cc. in order to maintain an adequate blood plasma vitamin A. Some individual variation was apparent in the amount of plasma carotene necessary to maintain an adequate plasma vitamin A content. The range for Holsteins was 50–70 $\gamma$  of carotene per 100 cc. and for Guernseys 110–140 $\gamma$  of carotene per 100 cc. These data were obtained when the calves had been on experiment for about one year. They were at the time somewhat undersized but otherwise good experimental animals.

#### SUMMARY

Studies have been made to determine the blood plasma concentrations and the intakes of carotene and vitamin A necessary for the growing calf.

The data obtained showed that the blood plasma vitamin A was a more delicate measure of the state of vitamin A nutrition in the calf than either growth or blood carotene. A blood plasma vitamin A level of 10 $\gamma$  or more per 100 cc. was found to be necessary for adequate vitamin A nutrition of the growing calf. Blood plasma vitamin A levels of 7–8 $\gamma$  per 100 cc. were borderline levels while values below this were definitely inadequate.

Daily intakes of vitamin A which would maintain deficient, borderline, and adequate concentrations of blood plasma vitamin A were found to be approximately 6, 12 and 18 $\gamma$  per kg. of body weight respectively. The daily carotene requirements necessary to maintain an adequate plasma vitamin A and prevent deficiency symptoms were 75 $\gamma$  per kg. for Holstein yearlings and 125 $\gamma$  per kg. for Guernsey yearlings.

The blood plasma carotene levels which would maintain an adequate blood vitamin A were 50–70 $\gamma$  of carotene per 100 cc. for Holsteins and 110–140 $\gamma$  of carotene per 100 cc. for Guernseys.

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## MINERALS IN DAIRY CATTLE NUTRITION: A REVIEW

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Different nutrients during past decades have held the spotlight, more particularly the proteins, minerals, and vitamins. Vitamins during the past twenty years have been stealing the scene from the minerals. Nevertheless, due in part to the promotion of mineral feeds, there is a concern in the minds of dairymen about possible mineral deficiencies among their cattle. Possibly the more or less constant need for salt and the chore of feeding salt have served as reminders that minerals other than salt may be necessary.

Since common methods of cropping and of pasturing land without compensatory returns tend to deplete the land and its products of certain minerals, it is not illogical to reason that this deficiency might be reflected in the well-being of animals subsisting on such pastures or hay crops. The animals might react all the more certainly if, meanwhile, as a result of a more or less consistent breeding program, they had been endowed with a strong growth impulse or milk-producing ability, and therefore might be making keener demands upon their rations. Nor would it seem that this picture is improved any by periodic occurrences of drought (8) making for poor phosphorus assimilation on the part of the growing vegetation. There may also be added the factor of unfavorable haying seasons, the shattering of leaves in the field, and the resulting coarse, perhaps discolored, musty hay. In view of these factors or possibilities, the concern of this owner or herdsman may, indeed, seem justified.

However, all things are relative. Whether there is a point to becoming alarmed over any serious situation facing the vast majority of dairymen may be seen from a survey of experimental findings.

Fortunately a number of long-continued critical mineral feeding experiments with dairy cows have been concluded during the past fifteen years. For the most part these experiments have focused on the elements calcium and phosphorus that make up by far the larger portion of the mineral composition of cows and of milk. Earlier feeding experiments related to salt and iodine, a few to sulphur. But more recent experiments, many of them growing out of observations in the field, have had to do with the trace mineral elements and with iron in combination with copper.

Very helpful in a study and appreciation of the mineral problem in dairy cattle and other livestock have been, among others, a number of relatively recent reports of original work, monographs, or bibliographies, by E. B. Forbes and co-workers (20), H. H. Mitchell and F. J. McClure (43), H. Schmidt (55), E. J. Underwood (59), and Muriel E. Whalley (64).

Underlying a consideration of mineral needs by ruminants especially is the status of the mineral composition of pastures and forage crops, on which subject J. B. Orr (47), and more recently Kenneth C. Beeson (8), have made noteworthy contributions. The comprehensive character of the work of these and others obviates the necessity for listing any considerable number of citations in the present limited space assigned to a consideration of minerals in dairy cattle nutrition.

The requirements for the several minerals by cattle usually are expressed in terms of percentage composition of their rations. Naturally the pounds of feed intake determine the pounds or ounces of mineral intake, and this quantitative concept usually is expressed as a specific daily mineral intake for a given live weight. But whether expressed in either one of those two terms, there is surprisingly little reference in the literature (37) to the considerable difference in digestibility or availability of calcium, phosphorus, etc., from perhaps milk, on the one hand, and alfalfa hay on the other, both feeds in varying proportions being fed in mineral feeding experiments with calves. Likewise grain and roughage may in varying proportions be fed in mineral feeding experiments with cows. Obviously, the calcium, phosphorus, or other mineral from milk or grain is more easily digested than are the corresponding minerals from the fibrous tissues of roughage. An assumption that minerals in feeds are digested in keeping with the digestibility of their dry matter would put a unit of minerals from roughages and a unit of minerals from concentrates more nearly on par.

Such variation in digestibility and in availability after having been digested, along with our limited understanding of the true requirements for minerals under various conditions, prompts a liberal margin of safety in the specifications for the several mineral elements in dairy cattle rations.

#### CALCIUM

Calcium is the major mineral element in respect to quantity contained in the bodies and in the milk of cows. For that reason and because the consequences of calcium deficiencies have been frequently observed, this element has challenged the attention of research workers for many years.

Reed and Huffman (50) in a 5-year experiment with different proportions of calcium in dairy rations made up of timothy hay, corn silage, and a grain mixture with no mineral supplement except salt, found this ration having 0.28 per cent calcium on the air-dry basis, adequate for normal growth, good reproduction, and a liberal milk flow.

Hart, Hadley, and Humphrey (26) in an experiment lasting 5 years and involving 22 cattle in each of two lots, found that only about 0.20 per cent calcium in the ration on the dry basis between pasture seasons permitted good health and, so far as the calcium question was uncomplicated by infection, permitted successful reproduction. The basal ration was

timothy hay, corn silage, and a grain mixture which was made up of corn, oats, corn gluten meal, and salt. The cows in the second group were fed alfalfa hay with corn silage and a grain mixture which consisted of corn, oats, wheat bran, linseed meal, bone meal, and iodized salt. In spite of having several times as much calcium as the first ration, the results were practically the same.

Fitch and co-workers (19) were able to reduce the calcium to 0.18 per cent of the entire ration on the dry matter basis while still obtaining good results in respect to reproduction, calcium content of the blood plasma, and milk and fat production. They state that the animals adjusted themselves to the calcium content of the ration and conserved the quantity ingested when it was limited. The basal ration was made up of timothy hay from acid soil, one per cent cod-liver oil in the grain mixture, and one pint daily of canned tomatoes. Several vitamins, including vitamin D, were therefore provided in relative abundance. Just how much of a saving feature these additions were, may be suggested by the work of Hart and associates (28) showing that the amount of calcium and phosphorus lost from the body of lactating cows was less when green grass was fed than when dry grass was given, and that it was also less when alfalfa hay properly cured in the sunlight was fed than when poorly cured alfalfa hay was fed (31).

To put this question of vitamins and availability of minerals to a further test, Palmer and co-workers (48) withheld the vitamin supplements and further reduced the calcium content of the ration to 0.12 per cent on the dry matter basis. They reported no abortions attributable to this low level of calcium during one or two succeeding gestations. Nor did this ration appear to have any effect upon the milk and butterfat production or the chemical composition or clotting of the milk. It did, however, slightly reduce the total and unfilterable calcium content of the blood plasma and it did lower the ash content of the bones.

The calcium content of milk seems to be independent of the calcium level of the ration (37). Milk secretion draws on the calcium reserves of the body and apparently ceases when the available material for it gives out.

For growth, more particularly for growth and fattening (62) where body reserves cannot be drawn upon as in the case of the cow, added calcium has been shown necessary with rations similarly constituted except for the relatively large proportion of corn or concentrate. With growing cattle also, sunlight or vitamin D which is supplied at times by sun-cured hay, plays a more important role than with mature animals (51). Early consumption of good quality sun-cured hay has been found (39) to obviate the necessity for vitamin A and D concentrate, or cod-liver oil additions.

The Minnesota work with the very low level of 0.12 per cent calcium in the ration of mature cows reveals a surprising adaptability by the animals to a calcium deficiency in the ration. While this low level brought on mildly



unfavorable responses, this experiment nevertheless is reassuring that the margin of safety in commonly used rations may be adequate. A level of 0.18 per cent calcium in experimental rations used by the same station gave good results in the several criteria used.

What does this mean in reference to the Kellner standard of calcium and phosphorus for maintenance and milk production? Kellner (37)

TABLE 1

*Testing practical American dairy rations against European mineral feeding standards*

	Kellner standard (7, 37)		Wellmann standard (7, 63)	
	Calcium (Ca)	Phosphorus (P)	Calcium (Ca)	Phosphorus (P)
	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
1000 #-cow, maintenance	.0715	.0218	.0358-.0716	.0131-.0218
1-pound gain in body weight			.0250	.0122
1 pound milk produced	.0018	.0011	.0036	.0020
<b>Standards for milking cow:</b>				
1000 #-cow giving 30 lb. milk	.1255	.0548	.1438-.1796	.0730-.0818
Ration* for above cow, using timothy				
hay, corn silage, grain	.0608	.0980	.0608	.0980
.16 lb. CaCO <sub>3</sub> with 10 lb. grain	.0647			
	.1255			
.21 lb. CaCO <sub>3</sub> with 10 lb. grain			.0830	
			.1438	
Therefore 1.6% or 2.1% CaCO <sub>3</sub> in grain mixture are needed to supply Ca.				
Ration† for above cow, using alfalfa				
hay, corn silage, grain	.1691	.0835	.1691	.0835
This practical ration is sufficiently rich in Ca and P to satisfy both standards.				
Above ration with bran omitted and				
correspondingly more oats	.1686	.0690	.1686	.0690
0.112 lb. bone meal with 10 lb.				
grain				.0145
				.0835
Without bran this ration needs 1.12% bone meal in the grain mixture to supply the same amount of P, but needs only 0.31% bone meal to meet the minimum P of standard				
<b>Standard for growing cattle:</b>				
500 #-heifer, maintenance			.0179-.0358	.0065-.0109
1.5-lb. daily gain			.0375	.0183
			.0554	.0248
Growing ration,‡ using timothy hay,				
grain			.0322	.0345
0.058 lb. CaCO <sub>3</sub> with 3.5 lb. grain			.0232	
			.0554	
Therefore 1.66% CaCO <sub>3</sub> in grain mixture is needed to supply Ca.				

\* Timothy hay 10 pounds, corn silage 30 pounds, grain mixture 10 pounds (corn 3.5, oats 2.0, wheat bran 2.0, linseed meal 2.5 pounds).

† Alfalfa hay 10 pounds, corn silage 30 pounds, grain 10 pounds (corn 5.5, oats 3.0, wheat bran 1.5 pounds).

‡ Timothy hay 10 pounds, grain mixture 3.5 pounds (corn 2, linseed meal 1.5 pounds).

specifies 100 grams CaO (71.5 grams Ca) and 50 grams  $P_2O_5$  (21.8 grams P) for the maintenance of 1000 kg. live weight, which therefore amounts to .0715 pound (32.5 grams) Ca and .0218 pound (10 grams) P for every 1000 pounds live weight. For every pound of milk produced, he adds to the above .0018 pound Ca and .0011 pound P.

According to the above Kellner standard, dairy rations made up of timothy hay, corn silage, and a grain mixture to balance the ration, using average figures for mineral composition (44), in the case of lactating cows would require about 2 per cent  $CaCO_3$  or high calcium ground limestone added to the grain mixture. More or less than that amount would need to be added depending on the amount of grain fed or the level of milk production. The Florida workers (7) compared the specifications of the Kellner and Wellmann standards for calcium and phosphorus, and these standards are made use of in table 1 for testing the adequacy for calcium and phosphorus of common American dairy rations.

It is seen that by these two European standards it would be necessary to add calcium to timothy hay and corn silage rations, using average figures of composition. Where legume hay makes up one-half or more of the roughage, the required calcium is well supplied.

However, dairy rations that have a large portion of the necessary protein furnished by legume roughage such as alfalfa or by one of the protein concentrates which is relatively low in phosphorus, such as corn gluten meal, barely satisfy the minimum phosphorus requirement of the Wellmann standard for either growth or milk production. They do meet the Kellner standard for phosphorus.

It is seen from table 1 that, considering average composition of a number of typical American feeds, there is according to both European investigators a shortage of calcium where grass hay and corn silage are the roughages in the ration. The amounts of  $CaCO_3$  necessary with such rations range from 1.6 to 2.1 per cent of the grain mixture, assuming more or less normal grain allowances in keeping with milk production. With legume hay and a modest proportion of protein concentrates in the grain mixture, the phosphorus requirement was met and the calcium requirement amply met. But without a protein concentrate it was necessary to add bone meal or other carrier of phosphorus to the ration in order to satisfy the standard.

In the light of American experience and the long-time feeding experiments in this country, which have been referred to, there is no need to fear a calcium deficiency in dairy rations except where timothy or grass hay, or low protein roughage in general has been grown on acid soil. The timothy or other grass would be still lower in calcium if cut at too mature a stage of growth and thereupon cured or stored under unfavorable conditions. A calcium deficiency is a relatively rare or regional problem (7). Kellner and Wellmann in retrospect seemed unduly concerned over this matter when they

hold that dairy rations are deficient in calcium more frequently than in phosphorus (63). If the old Wolff or the Wolff-Lehmann feeding standard for dairy cows may be taken as an example, the cattle in the particular European countries concerned seem all along to have been given generous allowances of oil meals, mill feeds, or other protein concentrates, which more or less automatically supply an abundance of phosphorus. Such European practices and experiences, therefore, would differ sharply from the experiences of cattle raisers on the South African veld or on some of our western ranges or other extensive cattle raising areas of the world where cattle grow and subsist very much more exclusively on range forage and a minimum of concentrates. Calcium is far from being the main concern of ranchmen or, for that matter, dairymen in this country.

A calcium deficiency may be a problem where non-legume roughage grown on rather unproductive acid soil (7) makes up the roughage of the ration. A relatively large intake of concentrates rich in phosphorus might then aggravate an imbalance of the two minerals involved. Or with a minimum amount of roughage of any sort and a maximum amount of grain and oil meal or mill feed, growing cattle may in time show a calcium deficiency.

#### PHOSPHORUS

Deficiencies of phosphorus among ruminants or herbivorous animals far outnumber deficiencies of calcium. As one evidence of this situation, most countries of the earth have certain names or designations for phosphorus deficiency diseases whereas they do not have a similar array of terms for calcium deficiency diseases. To list a few names for aphosphorosis: Pica, styfsiekte, loin disease, creeps, stiffs, sweeny, peg-leg, cripples. Theiler, Green, and du Toit (58) of South Africa, solved in a rather dramatic way the cause of lamsiekte, or bovine botulism, by tracing this pathogenic disease to a deficiency of phosphorus in the soil and therefore in the range forage. The disease was contracted by cattle having a depraved appetite, chewing carcasses and bones of cattle that had succumbed to the disease. In this way the living cattle contracted the disease because of their craving for phosphorus induced by a deficiency in the range forage growing on phosphorus-deficient soil. Feeding bone meal or other form of phosphorus prevented the disease.

Subsequently a number of phosphorus-deficient areas have been identified in many countries, including this country, in the states of Texas (53), Wisconsin (25), Minnesota (15), Pennsylvania (23), Florida (6), and a number of other states. As in South Africa (13) the administration of bone meal or other phosphate has cured or prevented the difficulty (54). While hardly feasible in the range country, it is entirely feasible and practical to apply phosphate fertilizer on the more intensively managed dairy farms of the country.

Pica or depraved appetite is prevalent where the soil is low in available phosphorus and where cattle subsist mainly or entirely on roughages as do range cattle or as may dairy cattle. The addition of grain, particularly protein concentrates, raises the phosphorus level in the ration considerably. But in the absence of such a protein concentrate some other phosphorus carrier like bone meal must be fed to assure a sufficiency of this element for growth, gestation, and milk production. The South African workers (9) have found it practicable to dissolve phosphates in drinking water for range cattle.

The necessity for added phosphorus was demonstrated at the Michigan Experiment Station by Huffman and co-workers (34) using a ration including alfalfa hay containing less than 0.20 per cent phosphorus, corn silage with a still lower, and corn with a somewhat higher phosphorus content. This ration of about 0.20 per cent phosphorus of the entire ration on the dry matter basis caused an immediate lowering of the inorganic blood phosphorus which is a satisfactory and early symptom of a phosphorus deficiency. The occurrence of this blood condition usually preceded anorexia. They did not find a depraved appetite a reliable index of phosphorus deficiency. The Michigan workers state that during the winter months when there are very few ultraviolet rays in sunshine, there may be interference with phosphorus utilization when the phosphorus level in the ration is low. In Pennsylvania (23) cases of phosphorus deficiency were found more frequently when cattle were wintered on cereal straw and corn stover. The investigators state that absence of sunshine "serves to aggravate the effect of a phosphorus deficiency."

These observations suggest that there may be a number of factors playing a part in phosphorus utilization, not merely the phosphorus content of the feed, but also digestion and assimilation which latter is also affected by parathyroid activity. Much of the phosphorus in grains and concentrates is in the form of phytin which in experimental rat feeding and with humans has not been metabolized very efficiently (38). Nevertheless in practical rations and in the presence of phytin-splitting enzymes in the blood and livers, this phosphorus compound has proved an efficient source of phosphorus for farm animals (29).

Huffman and associates (34) after extensive feeding experiments with various dairy rations supplemented and unsupplemented with phosphorus compounds, recommend that milking cows be provided with 10 grams of phosphorus (the Kellner standard) in their ration for every 1000 pounds live weight, that for every pound of milk produced they receive 0.75 gram phosphorus in their ration, and that not less than 17 grams phosphorus should be fed during low production and during the dry period. This amount of phosphorus would be amply supplied by common rations that are recognized as satisfactory, such as the rations for milking cows indicated in

the foot-note of table 1, which have .26 or .27 per cent phosphorus on the air-dry basis, where only about .24 per cent phosphorus in the ration as a whole would be necessary. Such rations, instead of 0.75 gram phosphorus, supply from about 0.9 to 1.1 gram phosphorus for each pound of milk in addition to maintenance. As milk production increases, the percentage of phosphorus must increase. Thus, where according to the above standard a 1200-pound cow giving 30 pounds of milk daily would need about 0.24 per cent phosphorus in her ration, if she gave 60 pounds of milk she would need about 0.30 per cent phosphorus in her ration, on the air-dry basis.

Obviously, if the ration consisted of alfalfa hay alone, which may have only about 0.21 per cent phosphorus, or if corn silage or beet pulp or limited amounts of corn were fed with it, this might bring on a phosphorus deficiency in milking cows. Practical ways of supplying phosphorus would be adding 1 or 2 per cent bone meal to the grain mixture, or having at least 20 per cent of such phosphorus-rich protein concentrates as wheat bran, cottonseed meal, or linseed meal in the grain mixture.

An uncomplicated phosphorus deficiency (16) was brought on by Minnesota workers feeding for 2 to 3 years a ration of prairie hay which on the average had only about 0.07 per cent phosphorus, and a grain mixture in which corn gluten meal was the only protein concentrate used. As a result of this ration, the inorganic phosphorus of the blood plasma of the cows fell to about one-half of the normal level, or to about 2.5 mgm. per cent, this response being an indication of aphosphorosis. This ration did not cause abnormal estrum although it did appear to reduce breeding efficiency.

Many times in the field a phosphorus deficiency is combined with a protein deficiency (49). Uncomplicated phosphorus deficiencies have delayed sexual maturity and have repressed evidences of estrum, but have not prevented ovulation and conception. They did not prevent normal vigor in new-born calves, though the dams were "undersized, miserable appearing specimens." Four out of 8 of the cows had difficulty in parturition.

#### THE RELATIONSHIP OF CALCIUM AND PHOSPHORUS

Calcium and phosphorus usually are discussed together because of their natural affinity throughout nature and in animal nutrition. However, in the nutrition of dairy cattle the two elements can be discussed independently more satisfactorily than in the nutrition of pigs or some other animals that subsist largely on grains or concentrates, especially if such animals should be deprived of vitamin D as supplied by outdoor sunshine or by its presence in the ration. This recognizes the partnership existing between the two mineral elements and vitamin D where the latter serves a corrective calcifying function when either or both of the two elements are present in sub-optimal amounts. In the absence of vitamin D, if it can be spared at all with some species of animals, the two elements must be present in nearly the right

amounts and proportions for optimum results. But with an abundant amount of vitamin D present, some rather wide departures from the optimum are permissible.

Possibly because of consuming liberal amounts of sun-cured hay, if they are not themselves exposed to outdoor sunlight (39), possibly because of species difference as a factor, cattle do not require a rigid Ca : P ratio in their ration. Rather it is necessary that either of the two minerals be present in at least minimum necessary amounts, then the body metabolism within rather wide limits takes care of the surplus amount of the other element. Nevertheless it has been suggested that from 1 to 2 parts of calcium to every 1 part of phosphorus is the desirable relationship. Ca : P ratios as extreme as 4 : 1 or wider have been fed to both growing (14) and mature (34) cattle with apparent success. Alfalfa hay thus has a ratio of about 7 : 1. Feeding alfalfa hay alone sets a rather low limit to milk production (33) unless the cows are fed bone meal or other source of phosphorus (24, 36). The feeding of most any grain or concentrate with alfalfa would narrow the ratio. In mineral balance studies at Vermont (18) cows could be fed large amounts of calcium where for some weeks the cows were on a negative calcium balance. Still there was no interference with the utilization of the phosphorus.

Cows are also rather insensible to exposure to sunlight or ultraviolet light in so far as redeposition of calcium and phosphorus or checking a negative balance is concerned (27, 30). When definitely rachitic (60), cows have proved responsive to vitamin D administration by various means. Also milk produced by cows on pasture, with the animals therefore exposed to an abundance of ultraviolet light, proved richer in vitamin D than milk produced during the winter when the cows were kept largely in the barn (12, 41, 61). The relative prevalence of milk fever during winter months when solar radiation is poorest may also indicate the existence of a greater responsiveness to sunshine on the part of the cows than has been held to be the case.

Calves tolerate a rather large proportion of calcium to phosphorus. At Wisconsin (51) calves grew better on a Ca : P ratio of 3 : 1 than 1.5 : 1. According to Sheehy and Senior (56), it is advisable to add calcium to the ration of calves when the Ca : P ratio is less than 1.64 : 1 and that by correcting the balance in this way the retention of phosphorus is raised. When poor hay was fed, cod-liver oil raised the retention of calcium and phosphorus, but was not otherwise necessary. Du Toit and co-workers (14) also found that a Ca : P ratio of 7.7 : 1 produced as good results with calves as one of 1.17 : 1. The practice of feeding lime in some form to calves may have a sound nutritional basis.

That cows during most of their lactation are not very acquisitive in respect to a number of nutrients, including minerals, was proved by the work of Forbes and associates (21, 22), and Ellenberger and associates (17, 18) with cows which during the flush of milk production were on a negative

calcium and phosphorus balance, and replenished their stores only during the drying off and the dry period. The cows obviously used their skeletons as a reserve for those minerals and doled them out to the milk. It has been stated that while the flood gates of milk production were open, it was difficult to push minerals upstream, as might be attempted by feeding added minerals in the ration.

This cycle is to be looked upon as a normal one which emphasizes the importance of the dry period. A cow at that time should store not merely visible reserves by way of body fat, but also invisible reserves, the minerals and vitamins. Too often a dry cow is looked upon as essentially in cold storage. But her ability to produce milk during the following lactation depends in large part on the quality of her feed, including minerals (1, 42).

Mineral feeding standards relating to calcium and phosphorus have been discussed and the mineral contents of rations that are practical in many dairy sections have been checked against them, showing that the European standards were rather high in their calcium specification. Dairy rations making extensive use of legume roughage in many cases seemed low in phosphorus, especially in high-producing cows. Considerable dependence naturally would be placed on long-time mineral feeding experiments. Such experiments have been conducted, among others, at Michigan (50), Ohio (32), Pennsylvania (4, 20), Massachusetts (40), and Wisconsin (26), all of them showing that with the feeding of rations that were practical on dairy farms of those states, no additional calcium or phosphorus was necessary. The herds of cows used on these experiments were for the most part such as would be representative of the better dairy herds of the region and did not include many high-producing cows. Such cows will probably always need special attention in respect to all of the nutrients.

Likewise in areas where through soil deficiencies or for weather or climatic reasons the roughages should be deficient in calcium, phosphorus, or other minerals, suitable additions to the ration may need to be made.

Where grasses or cereal forages constitute the roughage part of the ration, and where the grain mixture of necessity needs to be fortified with protein concentrates, a possible lime deficiency of the ration as a whole may need to be kept in mind, but such a deficiency seems surprisingly remote in the light of experimental findings in this country. However, forages grown on acid soils, particularly acid sandy soils, may present such a calcium problem.

Any time legumes make up a large part of the roughage, only phosphorus is likely to be deficient, and then only provided the roughage has been grown on phosphorus-deficient soil and is fed with a minimum amount of grain or concentrates. When such protein concentrates as wheat bran, linseed meal, and cottonseed meal are fed to the extent of 20 per cent of the grain mixture, and the grain mixture is fed in usual amounts in relation to milk production, phosphorus is well supplied.

In consideration of the findings at various American experiment stations, and mindful of earlier work abroad, Mitchell and McClure estimated mineral requirements of growing dairy heifers, gestating cows, and milking cows, as given in tables 2, 3, and 4. The computation of the percentages of the two mineral ingredients in the dry ration, which computation was derived from the "grams required intake," presupposes a certain feed intake for which the provisions of the Morrison feeding standards were used. A percentage relationship of total digestible nutrients to the dry matter of the ration was assumed and was used for all three tables in those columns in which the percentages calcium or phosphorus of the dry ration are indicated:

TABLE 2

*Estimated calcium and phosphorus requirements of growing Holstein-Friesian cattle (female)*

From H. H. Mitchell and F. J. McClure (43)

Body weight	Feed calcium required	Necessary percentage of calcium in dry ration	Feed phosphorus required	Necessary percentage of phosphorus in dry ration
<i>lbs.</i>	<i>grams</i>	<i>per cent</i>	<i>grams</i>	<i>per cent</i>
300	11.3	0.33	10.3	0.30
400	10.4	0.27	10.4	0.27
500	9.4	0.21	10.4	0.24
600	8.7	0.18	10.4	0.22
700	7.7	0.15	10.3	0.20
800	7.1	0.13	10.3	0.19
900	6.4	0.11	10.3	0.18
1000	5.9	0.10	10.3	0.17
1100	5.6	0.08	10.6	0.16
1200	5.4	0.08	10.9	0.16

If the provisions for a growing heifer or for a 1000-pound cow giving 30 pounds milk are checked against the corresponding provisions by Kellner and Wellmann in table 1, it will be seen that all of the unsupplemented rations would have satisfied this newer standard. This means in general that rations made up of feeds of average composition do not need added bone meal or calcium carbonate.

Table 2 shows that heifers which presumably were to be managed in a practical manner, having outdoor exercise and sunlight, should get along on a ration made up of commonly used feeds. At an early stage of growth the percentage of both calcium and phosphorus needs to be fairly high, as would be provided by using legume hay for at least part of the roughage and by using protein concentrates to balance the ration. Beyond about 600 pounds live weight a rather ordinary ration of roughage and grain supplies the necessary amounts of the two minerals.

Not, however, if this heifer should be in calf, for then according to table 3 both the calcium and phosphorus requirements increase quite rapidly toward the final months of gestation. The ration would need to include



TABLE 3

*Estimated calcium and phosphorus requirements of a pregnant Holstein-Friesian cow weighing 1000 pounds*

From H. H. Mitchell and F. J. McClure (43)

Month of gestation	Feed calcium required daily	Necessary calcium in dry ration	Feed phosphorus required daily	Necessary phosphorus in dry ration
	<i>grams</i>	<i>per cent</i>	<i>grams</i>	<i>per cent</i>
4	6.0	0.10	10.4	0.17
5	6.4	0.10	10.7	0.17
6	7.6	0.12	11.6	0.18
7	10.4	0.16	13.3	0.21
8	12.4	0.19	16.3	0.25
9	27.9	0.42	20.4	0.31
Average	11.8	0.18	13.8	0.22

the kind of roughage and grain or concentrate mixture that equals in quality, not in quantity, the rations fed to cows of good production.

The mineral specifications for milk production are given in table 4. It will be appreciated from the figures for calcium and phosphorus that, in the light of previous discussion of these two minerals, their necessary percentages in the ration are such as would be taken care of by common feeds of average composition. For the production of milk of varying fat content, allowances are made and larger amounts of calcium and phosphorus are specified for increasing richness of the milk in both butterfat and solids-not-fat.

TABLE 4

*Estimated calcium and phosphorus requirements of 1000 pound Holstein-Friesian cows producing varying amounts of 3.5 per cent milk*

From H. H. Mitchell and F. J. McClure (43)

Daily milk production	Feed calcium required daily	Necessary calcium in dry ration	Feed phosphorus required daily	Necessary phosphorus in dry ration
<i>lbs.</i>	<i>grams</i>	<i>per cent</i>	<i>grams</i>	<i>per cent</i>
10	12.7	0.16	15.7	0.20
20	19.6	0.21	21.1	0.23
30	26.4	0.24	26.6	0.25
40	33.3	0.27	32.0	0.26
50	40.1	0.29	37.4	0.27
75	57.3	0.32	51.0	0.29
100	74.4	0.35	64.6	0.30

In the light of the more or less generally accepted desirable Ca : P ratio of 1 to 2 : 1, the question may be raised why in every case except for the three conditions of early growth, the last stages of gestation, and high milk production, the Ca : P ratio is less than unity.

## OTHER MINERALS

Salt ( $\text{NaCl}$ ) is used almost universally in dairy cattle rations, and its importance has been duly recognized for ages. The need for salt was emphasized by an experiment by Babcock (2) who withheld salt from one-half of a dairy herd with the result that in time these animals presented a strikingly adverse contrast to the other half of the herd. The salt-starved cows ran down badly in condition, they decreased in milk production, and failed to deliver normal calves. Babcock's recommendation for feeding salt was to allow  $\frac{3}{4}$  ounce salt daily per 1000 pounds live weight and in addition  $\frac{3}{10}$  ounce salt for every 10 pounds of milk produced. Feeding the customary one per cent salt in the grain mixture and feeding grain in the usual proportion to milk provides most milking cows with enough salt during the larger part of the lactation period, but may not take care of their need for salt during the drying off or dry period, nor any time their grain allowance should be greatly restricted in favor of more roughage. A reasonable arrangement is to allow all members of the herd free access to salt in addition to any that may be mixed with the grain. Records kept of the salt consumption of individual cows have shown great differences (11) and suggest that the need for salt by individual cows differs greatly.

Iodine deficiencies appear to be confined to some of the northwest states and to the Great Lakes region. The statement that iodine makes for improvement in the nutrition of cattle in areas outside of these regions, has not been generally accepted. Where "big neck" calves have occasionally been born, iodine in some form should be fed, the most convenient method being the feeding of iodized salt.

Copper and iron (5) deficiencies in cattle have shown up in parts of Florida where cattle subsisted on forage grown on sandy soil which was extremely low in organic matter. Such occurrences may be found on other similarly constituted soil but need not prompt the practice of incorporating iron and copper in the rations of dairy cattle elsewhere.

A disease among cattle in the Grand Traverse region of Michigan, which disease for some time had been considered a phosphorus deficiency disease, during the past few years has been identified as due to a low level of cobalt in the roughages grown in that and some other counties bordering Lakes Michigan and Huron (3). Cobalt-deficient areas have been identified along the coastal plains from Texas to the Carolinas, including Florida (45). Australia and New Zealand stockmen and research workers have known of this disease for some years.

The question of a possible lack of manganese in dairy rations has been raised, especially by research workers in dairy cattle reproduction. It has been appreciated for some time that manganese plays a role in some of the phases of reproduction. It has been found by Michigan workers (52) that

feeds differ greatly in their manganese contents and that the factors of soil types and soil reactions, and maturity of crops greatly affect the level of manganese in those crops. The place of manganese and the question of any necessary fortification of manganese in dairy cattle rations await an answer.

Magnesium in sub-normal amounts or in an imbalanced relationship with other mineral elements seems to play a part in grass tetany, but the magnesium picture as yet is not clear.

#### PRACTICAL CONSIDERATIONS

From the foregoing discussion it appears that minerals in dairy cattle nutrition present a problem in areas where the soil is low in available mineral elements, in some cases being an acid sandy soil, in others a soil of various types that has been cropped without adequate restitution. Except in a range country where phosphating or other manner of fertilizing is impractical, the way out of most mineral deficiencies among cattle is to feed the necessary elements to the soil and thus indirectly to the animals subsisting on the products of the soil.

With rare exceptions feed crops, especially roughages that have been grown on fertile soil, are well supplied with those minerals of which the animal organism is directly in need. Thus the lime and phosphorus need of cows in liberal milk flow may be met by home-grown roughages and grains, especially when supplemented with such protein concentrates as may be necessary to balance the ration.

The ability of cows to store calcium and phosphorus in their skeletons against the day of need for milk production is reassuring. But a knowledge of this cycle in the physiology of cows should also be a warning to the owner to give cows a reasonably long dry period, and during that time to give them every chance to store the invisible reserves, the minerals and vitamins, as well as to put fat on their bodies.

Natural feeds that make up a ration are complex in their mineral composition and except for rare instances, or restricted geographical areas, satisfy the mineral requirement of cattle without the need of resorting to complex mineral mixtures. With the exception of salt, and in places iodine as may be supplied by iodized salt, the mineral which is most likely to be present in too small amounts in a dairy ration, is phosphorus. A calcium deficiency is rare and is almost precluded if any considerable proportion of the roughages is of a legume character.

This, essentially, appears to be the situation in respect to the need of dairy cattle for minerals. But adding minerals to dairy feeds is common practice. Also the mineral preparations, whether home-mixed or offered by the trade, usually contain from about 4 to 6 times as much calcium as phosphorus (10). Calcium obviously is many times cheaper than phosphorus

and this fact probably has a bearing on the situation. Reasoning from the facts presented, cows have a tolerance for a large amount of calcium and for a wide calcium: phosphorus ratio. They probably are not injured by a high intake of calcium in the form of legume hay plus additional ground limestone, or  $\text{CaCO}_3$ , except as suggested by Eckles and co-workers (15) when cattle are on sub-optimal intakes of phosphorus.

A sensible way of feeding minerals to dairy cattle is feeding about 1 per cent salt or iodized salt, if necessary, in the grain mixture and in addition, perhaps in a suitably protected box in the exercise yard or the pasture, some additional salt freely accessible to the animals. Then, if the owner should be worried about a possible deficiency, he might offer some bone meal or other suitable phosphate alongside the salt box. It may be desirable to mix from 10 to 20 per cent salt with the bone meal to make it more palatable, but the cattle should not be obliged to eat bone meal when they actually wish to eat salt. Where with the above arrangement (46) cattle have been given in addition free access to ground limestone, they have under normal conditions eaten extremely little limestone and only very little bone meal. Such has been common experience even where herds have consisted of medium to high producing cows.

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**PROGRAM**  
**THIRTY-SEVENTH ANNUAL MEETING**  
**OF THE**  
**AMERICAN DAIRY SCIENCE ASSOCIATION**

MICHIGAN STATE COLLEGE  
EAST LANSING, MICHIGAN

JUNE 22-25, 1942

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**PROGRAM COMMITTEE**

**MANUFACTURING**

E. H. PARFITT, Evaporated Milk  
Association (*Chairman*)  
P. F. SHARP, New York  
O. F. GARRETT, New Jersey

**EXTENSION**

J. F. KENDRICK, Bureau Dairy  
Industry (*Chairman*)  
E. C. SCHEIDENHELM, Michigan  
A. C. BALTZER, Michigan

**PRODUCTION**

H. A. HERMAN, Missouri  
(*Chairman*)  
W. E. PETERSEN, Minnesota  
K. L. TURK, Maryland

**GENERAL**

H. W. CAVE, Oklahoma (*Chairman*)  
E. H. PARFITT, Evaporated Milk  
Association  
H. A. HERMAN, Missouri  
J. F. KENDRICK, Bureau of Dairy  
Industry



## REGISTRATION

LOBBY—ABBOT HALL (*Men's Dormitory*)

Saturday 9:00 a.m.—1:00 p.m.

Sunday 2:00 p.m.—9:00 p.m.

Monday 8:00 a.m.—9:00 p.m.

Tuesday 8:00 a.m.—7:00 p.m.

Wednesday 8:00 a.m.—5:00 p.m.

Thursday 8:00 a.m.—12:00 m.

## COMMITTEE MEETINGS

## ROOM ASSIGNMENTS AND TIME OF MEETINGS

The Breed's Relations Committee is requested to meet at 8:00 A.M. on Tuesday, June 23, in Room 235, Abbot Hall.

The following committees are requested to meet at 11:00 A.M. on Tuesday, June 23, in the rooms as indicated—all in Abbot Hall:

Room 35—Feeds Specifications. E. S. SAVAGE, *Chairman*.

Room 236—Measuring Results of Pasture Investigations. G. BOHSTEDT, *Chairman*.

Room 335—Silage Methods, Evaluation, etc. C. B. BENDER, *Chairman*.

Room 336—Rules for Dairy Cattle Judging Contest. I. W. RUPEL, *Chairman*.

Grill—Awards for Dairy Cattle Judging Contest. A. A. BORLAND, *Chairman*.

The following are proposed as suitable rooms for other groups which may desire to meet. Reservation of rooms should be made with C. F. Huffman.

## In Abbot Hall:

Room 135—(Manufacturing) Quality of Milk and Milk Products.

Room 136—(Manufacturing) Methods for Analysis of Milk and Dairy Products.

Library—Board of Directors.

Lower lounge—Other Production Section committees.

Play room—Extension Section committees.

## In Mason Hall:

Lower lounge—Other Manufacturing Section committees.

## SCHEDULE OF PROGRAM (Eastern War Time)

Time	General	Extension Section	Production Section	Manufacturing Section
<i>Mon., June 22</i> 12: 00  1: 30- 3: 30  Evening 8: 00	Lunch, Parch- ment, Michigan Tour of KVP plant Committees			
<i>Tues., June 23</i> 9: 00-11: 00 11: 00-12: 00 12: 00- 1: 30 1: 30- 4: 00 4: 00- 6: 00  Evening 9: 00	Opening session Committees Barbecue   Reception and Entertainment	Committees  Joint symposium Tour of experimental barn and pastures	Committees  Symposium Judging dairy products (milk)	Committees  Symposium Judging dairy products (milk)
<i>Wed., June 24</i> 9: 00-11: 00  11: 00-11: 45 11: 45-12: 00 1: 30- 3: 30  3: 30- 4: 30 4: 30- 6: 00  6: 00 Evening 8: 00	Committees Group Picture    Fish Fry Entertainment, Don Cossack Male Chorus	Business and papers Committees  Papers  Business Demonstration of practices in mastitis control - main dairy barn	Papers— Div. A, B Committees  Papers— Div. A, B Business Business Demonstration of practices in mastitis control - main dairy barn	Papers  Committees  Symposium  Business Judging dairy prod- ucts (ice cream)
<i>Thurs., June 25</i> 9: 00-11: 00 11: 00-12: 00 1: 30- 3: 30  3: 30- 5: 00 Evening 6: 30	    Business Association banquet	Joint symposium Business Exhibits and papers	Business  Joint symposium	Papers Business Joint symposium
<i>Fri., June 26</i> 9: 00  11: 00 12: 30 2: 00	Post-convention trip, Dearborn, Michigan (tentative) Visit Ford Museum Luncheon Visit Green- field Village			

## PROGRAM FOR WOMEN

*Monday, June 22*

- 12:00 Luncheon at K.V.P. Community House, Parchment, Michigan. *Compliments of Kalamazoo Vegetable Parchment Company.*
- 1:30 Tour of K.V.P. Plant. *(Those not traveling by car will have convenient bus connections to East Lansing.)*

*Tuesday, June 23*

- 12:00 Barbecue—*Compliments of Ayrshire Breeders' Association and Michigan State College.*
- 3:00 Tea and Social Gathering.
- Evening 9:00 Reception and Entertainment.

*Wednesday, June 24*

- 9:30 Tour Beal Botanical Gardens.
- 10:30 Tour Forestry Nursery.
- 3:00 Demonstration—Floral Table Arrangements.
- 6:00 Fish Fry—Abbot Hall.
- Evening 8:00 Entertainment—Don Cossack Male Chorus, Main Auditorium.

*Thursday, June 25*

- 1:00 Luncheon and Bridge.
- 6:30 Banquet—Ballroom, Union Building.

*Friday, June 26*

- 9:00 Post Convention Trip, Dearborn, Michigan (subject to final decision regarding opening of Greenfield Village in 1942).
- 11:00 Visit to Ford Museum—*Admission, Compliments of Borden Farm Products Company.*
- 12:30 Luncheon—*Compliments of National Dairy Products Corporation.*
- 2:00 Visit Greenfield Village—*Admission, Compliments of Borden Farm Products Company.*

Women are particularly invited to attend the opening session of the General Program. They also will be welcome at any of the Section Programs.

## FOR THE CHILDREN

Supervised tours, picnics, swimming, canoeing, tennis, playground and other entertainment.

## GENERAL PROGRAM

*(Eastern War Time)**Monday, June 22*

- 12: 00            **Lunch at K.V.P. Community House**, Parchment, Michigan  
(north edge of Kalamazoo). *Compliments of Kalamazoo  
Vegetable Parchment Company.*
- 1: 30- 3: 30    **Tour of K.V.P. Plant.** (Those not traveling by car can have  
convenient bus connections to East Lansing.)
- Evening 8: 00   **Committees.**

*Tuesday, June 23*9: 00-11: 00    **OPENING SESSION. FAIRCHILD THEATER.**

**Call to Order**—EARL WEAVER, *Head, Department of Dairy Husbandry,  
Michigan State College.*

**Introductions**—**Officers of American Dairy Science Association.** H. F.  
JUDKINS, *President.*

**Past Presidents.** H. P. DAVIS, *Vice-President.*

**Past Directors.** R. B. STOLTZ, *Secretary.*

**Address of Welcome**—J. A. HANNAH, *President, Michigan State College.*

**Response and Address**—H. F. JUDKINS, *President, American Dairy  
Science Association.*

**The Foster Mother**—O. E. REED, *Chief, Bureau of Dairy Industry.*

**Announcements.**

- 11: 00-12: 00   **Committees.**
- 12: 00- 1: 30   **Barbecue**—*Compliments of Ayrshire Breeders' Association  
and Michigan State College.*
- Evening 9: 00   **Reception and Entertainment**—Lobby, Abbot Hall.

*Wednesday, June 24*

- 11: 00-11: 45   **Committees.**
- 11: 45-12: 00   **Group Picture.**
- Evening 6: 00   **Fish Fry**—Abbot Hall.
- 8: 00            **Entertainment**—Don Cossack Male Chorus, Main Audi-  
torium.

*Thursday, June 25*

- 3: 30- 5: 00    **Business Session**—Fairchild Theater.
- Evening 6: 30   **Annual Association Banquet**—Presentation of Borden  
Awards, Ballroom, Union Building.

*Friday, June 26*

- 9:00        **Post-Convention Trip**, Dearborn, Michigan (subject to final decision regarding opening of Greenfield Village in 1942).
- 11:00       **Visit Ford Museum**—*Admission, Compliments of Borden Farm Products Company.*
- 12:30       **Luncheon**—*Compliments of National Dairy Products Corporation.*
- 2:00        **Visit Greenfield Village**—*Admission, Compliments of Borden Farm Products Company.*

## SECTIONAL PROGRAMS

### EXTENSION SECTION

*June 22-25*

**Exhibits**—Display of Extension Teaching Ideas, Playroom, Abbot Hall

*Tuesday, June 23*

1:30-4:00 P.M.—Lower Lounge, Abbot Hall

PAUL PHILLIPS, *Chairman*

### *Symposium*

#### **Nutrition and Reproduction in Dairy Cattle.**

*Joint session of Extension and Production Sections.*

- A—The role of minerals in reproduction. R. B. Becker, University of Florida.
- B—Vitamin E and reproduction. H. B. Thomas, Iowa State College.
- C—Vitamin A and its relationship to reproduction with special reference to cattle. T. S. Sutton, Ohio State University.
- D—The role of Vitamin C in reproduction. H. A. Lardy, University of Wisconsin.

4:00-6:00 P.M.

Tour of Experimental Barn and Pastures—Experimental Barn  
Judging Dairy Products (Milk)—Room 211, Dairy Building

*Wednesday, June 24*

9:00-11:00 A.M.—Playroom, Abbot Hall

GLEN W. VERGERONT, *Chairman*

### *Business Session*

Announcements and appointment of committees.

*Testing Committee Report*

R. W. DICKSON, *Chairman of Committee (In charge)*

- A—Maintaining qualified tester personnel. A. J. Cramer, University of Wisconsin.
- B—Supervisory problems. E. H. Loveland, University of Vermont.
- C—Emergency adjustment in D.H.I.A. procedure. C. R. Gearhart, Pennsylvania State College.
- D—Current developments affecting D.H.I.A. J. B. Parker, U. S. Bureau of Dairy Industry.
- E—Recommendations of Committee.

*Sire Committee Report*

E. J. PERRY, *Chairman of Committee (In charge)*

- A—Interpreting and using proved-sire data effectively. R. G. Connelly, Virginia Polytechnic Institute.
- B—Present day techniques of artificial insemination. Geo. W. Trimberger, University of Nebraska.
- C—Storing, packaging and shipping semen. Leland Lamb, American Dairy Cattle Club.
- D—Recommendations of Committee.

*Dairy Cattle Health Committee Report*

GEO. E. TAYLOR, *Chairman of Committee (In charge)*

- A—Recommendations of Committee.

1:30–3:30 P.M.—Playroom, Abbot Hall

*Feeding Committee Report*

C. L. BLACKMAN, *Chairman of Committee (In charge)*

- A—Simple vs. complex rations for dairy cattle. C. F. Monroe and W. E. Krauss, Ohio Experiment Station.
- B—Recommendations of Committee.

*Dairy Farm Records Committee Report*

L. G. GILMORE, *Chairman of Committee (In charge)*

- A—Recommendations of Committee.

*Type Rating Committee Report*

J. W. LINN, *Chairman of Committee (In charge)*

- A—Recommendations of Committee.

*4-H Dairy Club Committee Report*

H. A. WILLMAN, *Chairman of Committee (In charge)*

- A—Recommendations of Committee.

*Quality and Marketing Committee Report*

EVERT WALLENFELDT, *Chairman of Committee (In charge)*

- A—The need for quality improvement. H. R. Searles, University of Minnesota.
- B—Methods and organization of dairy manufacturing extension. C. J. Babcock, U. S. Bureau of Dairying.
- C—Dairy manufacturing activities devoted to national defense. J. M. Jensen, Michigan State College.
- D—Recommendations of Committee.

*Business Session*

3:30–4:30 P.M.—Playroom, Abbot Hall

4:30–6:00 P.M.

- A—Demonstration of practices in mastitis control—Main Dairy Barn
- B—Judging dairy products (ice cream)—Room 211, Dairy Building

*Thursday, June 25*

9:00–11:00 A.M.—Lower Lounge, Abbot Hall

*Symposium***Input as Related to Output in Milk Production**

See Production Section program.

11:00–12:00 M.—Playroom, Abbot Hall

GLEN W. VERGERONT, *Chairman*

*Business Session*

1:30–3:30 P.M.—Playroom, Abbot Hall

*Exhibit Committee Report*

C. A. HUTTON, *Chairman of Committee (In charge)*

- A—Discussion of exhibits.
- B—Recommendations of Committee.

**PRODUCTION SECTION***Tuesday, June 23*

1:30–4:00 P.M.—Lower Lounge, Abbot Hall

*Symposium***Nutrition and Reproduction in Dairy Cattle (See Extension Section)**

4:00–6:00 P.M.—Experimental Barn

*Tour of Experimental Barn and Pastures*

*Wednesday, June 24*

9:00–11:00 A.M.—See Divisions A and B

1:30–3:30 P.M.—See Divisions A and B

3:30–4:30 P.M.—Lower Lounge, Abbot Hall

*Business Session*

4:30–6:00 P.M.—Main Dairy Barn

*Demonstration of Practices in Mastitis Control*

*Thursday, June 25*

9:00–11:00 A.M.—Lower Lounge, Abbot Hall

K. L. TURK, *Chairman*

*Symposium***Input as Related to Output in Milk Production.**

Joint session of Production and Extension Sections.

A—Results of cooperative experiments to determine input-output relationships in milk production—

Einar Jensen, U. S. Bureau of Agricultural Economics

T. E. Woodward, U. S. Bureau of Dairy Industry

B—Practical application of the experimental results—

Discussion (Brief reports by cooperating stations)

A. E. Tomhave, Delaware; J. H. Hilton, Indiana;

K. L. Turk, Maryland; R. E. Horwood, Michigan;

J. S. Moore, Mississippi; C. B. Bender, New Jersey;

A. C. Dahlberg, New York (Geneva); A. A. Borland, Penn.;

T. M. Olson, South Dakota; A. D. Pratt, Virginia.

C—What new knowledge has been contributed by the input-output investigations.

F. B. Morrison, Cornell University.

D—Discussion.

*Business Session*

11:00–12:00 M.—Lower Lounge, Abbot Hall

*Business Session*

1:30–3:30 P.M.—Lower Lounge, Abbot Hall

H. P. DAVIS, *Chairman*

*Symposium*

**Curricula**—Joint session with Manufacturing Section.



## PRODUCTION SECTION—DIVISION A

*Wednesday, June 24*

9:00–11:00 A.M.—Lower Lounge, Abbot Hall

H. A. HERMAN, *Chairman**Nutrition and Herd Management*

- P1—Improving dairy cattle pastures. W. B. Nevens, University of Illinois.
- P2—The ability of yearling heifers to withstand cold temperatures. J. R. Dice, North Dakota Agricultural Experiment Station.
- P3—Resting maintenance cost in growing dairy cattle. Samuel Brody, University of Missouri.
- P4—Occurrence and importance of still unidentified nutrients in milk and milk products. A. M. Hartman and C. A. Cary, Bureau of Dairy Industry, U.S.D.A.
- P5—Hydroxyamino acids in milk proteins. B. H. Nicolet, L. A. Shinn and L. J. Saidel, Bureau of Dairy Industry, U.S.D.A.
- P6—Utilization of urea by calves less than four months of age. J. K. Loosli, C. M. McCay, and L. A. Maynard, Cornell University.
- P7—The feeding value of Korean lespedeza seed as a protein supplement for milk production. H. A. Herman and A. C. Ragsdale, University of Missouri.
- P8—The biological values of Korean lespedeza, alfalfa, corn, and milk proteins for growing dairy heifers. Eric W. Swanson, H. A. Herman, and A. C. Ragsdale, University of Missouri.
- P9—A study of the nutritive value of some of the end-products of carbohydrate fermentation in the ensiling process. T. B. McManus and C. B. Bender, New Jersey Agricultural Experiment Station.
- P10—Ruminal gases in normal and bloated animals. T. M. Olson, South Dakota Agricultural Experiment Station.
- P11—The effect, on the butterfat percentage, of feeding moderate quantities of cod-liver oil. H. T. Converse and Rowland Trimble, Bureau of Dairy Industry, U.S.D.A.
- P12—Further nutritional studies on calf scours. Norman S. Lundquist and Paul H. Phillips, University of Wisconsin.

1:30–3:30 P.M.—Lower Lounge, Abbot Hall

*Vitamins and Reproduction*

- P13—Factors affecting the vitamin A and D potency of alfalfa hay. G. C. Wallis, South Dakota Agricultural Experiment Station.
- P14—The vitamin A and carotene content of the blood plasma of calves from birth to four months of age. L. A. Moore, Maryland Agricultural Experiment Station.

- P15—Vitamin C in dairy cattle nutrition. G. C. Wallis, South Dakota Agricultural Experiment Station.
- P16—Carotene (provitamin A) requirements of dairy cattle for conception. A. H. Kuhlman and W. D. Gallup, Oklahoma A. and M. College.
- P17—The relation of nutrition to breeding performance in dairy bulls. I. R. Jones, Oregon State College.
- P18—Some preliminary results of feeding chloretone to bulls. E. C. Scheidenhelm, A. L. Bortree, C. F. Huffinan, and C. F. Clark, Michigan State College.
- P19—The effect of amphyl on bull sperm. H. O. Dunn, C. E. Shuart, and O. F. Garrett, New Jersey Agricultural Experiment Station.
- P20—The relation of morphology to fertility in bull semen. G. W. Trimberger and H. P. Davis, University of Nebraska.
- P21—Studies of respiration rate of dairy bull spermatozoa. Ray E. Ely, University of Missouri.
- P22—The breeding efficiency of dairy bulls used both artificially and naturally. E. R. Berousek, University of Missouri.
- P23—A comparison of artificial vs. natural service in heifers when bred to the same sire. C. E. Shuart, O. L. Lepard, and J. W. Bartlett, New Jersey Agricultural Experiment Station.
- P24—Availability of carotene in alfalfa hay as compared with carotene in oil. J. H. Hilton, J. W. Wilbur, R. G. Westfall, and S. M. Hauge, Purdue University.

## PRODUCTION SECTION—DIVISION B

*Wednesday, June 24*

9:00–11:00 A.M.—Grill, Abbot Hall

K. L. TURK, *Chairman**Endocrinology and Milk Secretion*

- P25—The cause of the initiation of lactation at parturition. J. Meites and C. W. Turner, University of Missouri.
- P26—Prehypophyseal hormone (Mammogen) control of mammary development. E. T. Gomez, Bureau of Dairy Industry, U.S.D.A.
- P27—The effect of adrenalectomy on the lactogenic hormone and the initiation of lactation. J. J. Trentin and J. Meites, University of Missouri.
- P28—The influence of thyroxin upon the stimulation of mammary lobule-alveolar growth. John P. Mixner, University of Missouri.
- P29—The effect of thyroxin on rate of growth and efficiency of weight increment. Marvin Koger and C. W. Turner, University of Missouri.
- P30—Growth and energy metabolism of thyroidectomized cattle. Samuel Brody, University of Missouri.

- P31—The effect of thyrolactin on milk production, metabolism, and growth. E. P. Reineke, University of Missouri.
- P32—The chemical formation of highly active thyroprotein. E. P. Reineke, University of Missouri.
- P33—Methods of prolactin assay, including data on the prolactin content of the anterior lobe of beef and dairy cattle and female rabbits in several physiological conditions. S. R. Hall, Bureau of Dairy Industry, U.S.D.A.
- P34—An intravenously active ovulating factor in the juice of corn and oat plants. J. T. Bradbury and R. E. Hodgson, Bureau of Dairy Industry, U.S.D.A.
- P35—Further evidence of the existence and the physiological action of an orally active factor(s) in plant juices which affect the development of the sex organs of the rat. E. T. Gomez, Bureau of Dairy Industry, U.S.D.A.
- P36—Some possibilities for the use of diethylstilbestrol in dairy cattle. Arthur A. Lewis, University of Missouri.

1:30-3:30 P.M.—Grill—Abbot Hall

*Milk Secretion and Mastitis Control*

- P37—The influence of ascorbic acid on the gonadotropic content of the male rat pituitary gland. R. P. Reece and E. J. Weatherby, New Jersey Agricultural Experiment Station.
- P38—Vitamin D, the parathyroid glands, and calcium metabolism. I. L. Campbell, University of Missouri.
- P39—Oxygen uptake and CO<sub>2</sub> elimination of the bovine mammary gland. W. E. Petersen and J. C. Shaw, University of Minnesota.
- P40—The utilization of lactic acid by dried bovine mammary gland tissue. Phillip L. Kelly, University of Arkansas.
- P41—The effect of continued injection of pitocin upon milk and fat production. C. B. Knodt and W. E. Petersen, University of Minnesota.
- P42—The incidence and control of milk fever. C. F. Monroe, W. E. Krauss, T. S. Sutton, and W. D. Pouden, Ohio Agricultural Experiment Station.
- P43—The blood picture in normal and milk fever cows. W. E. Krauss, C. F. Monroe, R. G. Washburn, J. W. Hibbs, T. S. Sutton, and N. Van Demark, Ohio Agricultural Experiment Station.
- P44—The nature of the material in mastitic milk responsible for the White-side reaction. H. O. Dunn, J. M. Murphy, and O. F. Garrett, New Jersey Agricultural Experiment Station.
- P45—The value of tyrothricin (gramicidin) in a herd mastitis control pro-

gram. C. S. Bryan, Russell E. Horwood and C. F. Clark, Michigan State College.

P46—Experiences with lacto vaccine in the control of mastitis. C. F. Clark, C. S. Bryan, and Russell E. Horwood, Michigan State College.

P47\*—Mastitis and herd practices in the college dairy herd. Russell E. Horwood, C. F. Clark, and C. S. Bryan, Michigan State College.

(\*4:30–6:00 P.M. Demonstration of practices in mastitis control—Main Dairy Barn)

#### MANUFACTURING SECTION

*Tuesday, June 23*

1:30–4:00 P.M.—Lower Lounge, Mason Hall

O. F. GARRETT, *Chairman*

#### *Symposium*

#### **Problems in Dairy Manufacturing Due to the War**

A—The dairy industries position in W.P.B. Clyde Beardslee, Chief, Dairy Division, War Production Board.

B—The position of the dairy equipment manufacturers. Roberts Everett, Executive Secretary, Dairy Industries Supply Association.

C—The position of the public health service. A. W. Fuchs, Commander, U. S. Public Health Service.

D—Substitutes: types, kinds and application. G. W. Putnam, Vice President, Creamery Package Manufacturing Company.

E—Modifications in processing. P. H. Tracy, Department of Dairy Husbandry, University of Illinois.

4:00–6:00 P.M.—Room 211, Dairy Building

*Judging Dairy Products (Milk)*

*Wednesday, June 24*

9:00–11:00 A.M.—Lower Lounge, Mason Hall

L. H. BURGWARD, *Chairman*

#### *Bacteriology—Chemistry*

M1—The effect of acidity and temperature on the growth of *Oospora lactis* cultures. E. R. Garrison, University of Missouri.

M2—Various treatments which affect the growth of mold mycelia in cream and resultant butter. J. E. Edmondson and W. H. E. Reid, University of Missouri.

M3—The development of a positive phosphatase test on refrigerated pas-

- teurized cream. F. W. Barber and W. C. Frazier, University of Wisconsin.
- M4—The keeping quality of cream pasteurized at 165° F. for 30 minutes, variously treated, and stored at 0° F. E. S. Guthrie, Cornell University.
- M5—The keeping quality of unsalted butter made from sweet cream pasteurized at 165° F. for thirty minutes and stored at 0° F. and 32° F. C. N. Stark, E. S. Guthrie and J. J. R. Campbell, Cornell University.
- M6—Some observations concerning the ascorbic acid content of evaporated milk. D. V. Josephson and F. J. Doan, Pennsylvania State College.
- M7—Control and verification of vitamin D in milk. M. J. Dorcas, National Carbon Company, Inc., Chicago.
- M8—A voltammetric method for measuring the concentration of dissolved oxygen in dairy products. G. H. Hartman and O. F. Garrett, Rutgers University.
- M9—Studies of the mechanisms of oxidized flavor. W. Carson Brown and F. C. Olsen, West Virginia University.
- M10—Relation of dissolved oxygen to certain oxidation reactions in milk. G. H. Hartman and O. F. Garrett, Rutgers University.
- M11—The role of the oxidase producing bacteria in the development of oxidized flavor in milk. J. Frank Cone and C. J. Babcock, Bureau of Dairy Industry, Washington.
- M12—Bacteriological studies on creamery water supplies. R. T. Corley and B. W. Hammer, Iowa State College.

1:30–3:30 P.M. Lower Lounge, Mason Hall

W. H. E. REID, *Chairman*

*Symposium*

**Problems in the Securing of Milk Quality**

- A—As accomplished by state programs,  
 California. O. A. Ghiggoile, Chief, Bureau of Dairy Service, California Department of Agriculture.  
 Wisconsin. L. G. Kuenning, Chief, Dairy Division, Wisconsin Department of Agriculture.  
 Tennessee. V. L. Fuqua, Dairy Commissioner, Tennessee Department of Agriculture.
- B—As accomplished by cleaning methods,  
 Selection of detergents. L. H. Minor, J. B. Ford Company (Tentative).  
 Special methods and materials. L. Shere, Diversey Corporation.

3:30–4:30 P.M. Lower Lounge, Mason Hall

*Business Session*

4:30–6:00 P.M. Room 211, Dairy Building  
*Judging Dairy Products (Ice Cream)*

*Thursday, June 25*

9:00–11:00 A.M.—Lower Lounge, Mason Hall

R. WHITAKER, *Chairman*

*Dairy Products*

- M13—Sensory adaptation as a factor in the judging of dairy products for flavor. S. T. Coulter, University of Minnesota.
- M14—A quick, colorimetric method for estimating the quality of butter. E. S. Guthrie and Georges Knaysi, Cornell University.
- M15—The use of surface-active substances in the reconstruction of milk and cream. G. A. Richardson, University of California.
- M16—Forewarming temperature of plain condensed skim milk and properties of the resulting ice cream. Jack B. Clinch and J. H. Erb, Ohio State University.
- M17—Relation of different mix compositions and methods of processing to the texture, structure and stability of ice cream. C. W. Decker and W. H. E. Reid, University of Missouri.
- M18—The gases evolved by cheddar and limburger cheese. A. C. Dahlberg and F. L. Dorn, New York State Agricultural Experiment Station.
- M19—The preparation of crystalline rennin. C. L. Hankinson, Carnation Company, Milwaukee, Wisconsin.
- M20—The use of rennet paste in Romano-type cheese. C. A. Phillips, G. A. Richardson, and N. P. Tarassuk, University of California.
- M21—Studies relating to the canning of pasteurized milk cheese. A. C. Dahlberg and J. C. Marquardt, New York State Agricultural Experiment Station.
- M22—Comparative studies on cheddar cheese prepared with starter and with certain pure cultures. D. D. Deane and T. G. Anderson, Pennsylvania State College.
- M23—General action in cheese of an enzyme preparation from chicken stomach. F. J. Babel, G. F. Stewart, and B. W. Hammer, Iowa State College.

11:00–12:00 A.M.—Lower Lounge, Mason Hall

*Business Session*

1:30–3:30 P.M. Lower Lounge, Abbot Hall

H. P. DAVIS, *Chairman*

*Symposium*

**Curricula**—Joint session with Production Section.



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## A STUDY OF THE COLIFORM GROUP IN ICE CREAM<sup>1</sup>

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The purpose of these studies has been to ascertain the prevalence of members of the coliform group in typical commercial ice cream, and to determine the species or varieties present.

No attempt will be made to review the literature on this group as it is extensive and has been covered adequately in a number of recent papers.

### EXPERIMENTAL METHODS

Factory-packed samples of ice cream were obtained from fourteen ice cream manufacturers located in Minnesota. Samples consisted of vanilla, chocolate, and strawberry ice cream, and sherbet (or ice), in pint-size packages and "Cheerios." These samples were collected at the plants and were packed in dry ice while being transported to the laboratory.

Scoop, or dipper samples of ice cream were obtained from thirty retail stores in the vicinity. These samples were collected in sterile pint fruit jars, using the dealer's scoop or dipper, and were carried in an iced sample case. These samples were purchased at drug store fountains, confectioneries, ice cream shops, etc.

Samples received from the plants were removed from the original package and placed in sterile pint fruit jars just prior to analysis. These samples and also the scoop samples were melted in a water bath, kept at 45° C. (113° F.), for 15 minutes (8). Occasional agitation of the sample during melting helped to expel the air.

The presumptive media used in this work consisted of brilliant green-lactose-peptone-bile 2 per cent and violet red-bile agar.

The brilliant green-lactose-bile broth was made up and employed according to the directions given in "Standard Methods" (2), with the exception that a dye concentration of 1:30,000 of brilliant green as recommended by McAuliffe and Farrell (15) for milk was used instead of 1:75,188.

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<sup>1</sup> Paper No. 1962, Scientific Journal Series, Minnesota Agricultural Experiment Station.

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A buffered, brilliant green-lactose-bile medium containing 0.5 per cent of  $K_2HPO_4$ , was also used for sherbets and ices.

An estimation of the number of coliform organisms was made by using the dilution technique of Halvorson and Ziegler (9). For factory-packed ice cream, six dilutions were made with 10 tubes inoculated in each dilution. Volumetric measurements of the melted ice cream samples were made throughout this work (7). The largest inoculum, consisting of 10 milliliters of the sample, was planted into 100 milliliters of the brilliant green-lactose-bile broth. The other inocula, one milliliter to 0.0001 milliliter, inclusive, were planted into 10 milliliter quantities of the medium. However, in the case of the buffered and unbuffered brilliant green-lactose-bile broths only four dilutions were made, the inocula being 10 milliliters to 0.01 milliliter, inclusive, where five tubes each of the buffered and unbuffered medium were used in each dilution. In the case of the "Cheerios" the 10-milliliter inoculum was not used, the others being one milliliter to 0.0001 milliliter, inclusive.

The range of dilutions of inocula of the scoop samples was between one milliliter and 0.000001 milliliter, inclusive, where 10 tubes were used for each dilution.

After 48 hours' incubation at 37° C. all tubes were examined for gas production. Halvorson and Ziegler's tables (11) giving the most probable number of bacteria per milliliter were used for interpreting the data.

Direct counts of coliform organisms were made on violet red-bile agar plates that had been incubated 18–24 hours at 37° C.

Levine's eosin-methylene blue (E.M.B.) agar plates were streaked from tubes of brilliant green-lactose-bile broth of the lowest and highest dilutions showing gas after 24 and 48 hours. (Usually two tubes of each of the two dilutions were taken.)

Coliform-like colonies were picked from positive violet red-bile agar (V.R.B.) plates and were transferred to nutrient agar slants and later the cultures were streaked on E.M.B. agar plates.

Representative, typical and atypical, coliform colonies and non-coliform colonies were picked from streaked E.M.B. agar plates and were transferred to nutrient agar slants. The differentiation of *Escherichia coli* and *Acrobacter aerogenes* was made according to the suggestion of Levine (12) on eosin-methylene blue agar.

For purification, light suspensions were made in tubes of sterile distilled water from cultures growing on nutrient agar slants, and inocula from these suspensions were streaked on E.M.B. agar. After incubation, 24–48 hours, representative colonies of different types were picked and transferred to nutrient agar slants. This process was repeated so that three E.M.B. agar plates were streaked and colonies were transferred to three agar slants.

Gram and flagella stains and determination of motility were made from

nutrient agar slant cultures that had been incubated for 18–22 hours at 37° C. Hucker's modification (18) of the Gram stain was used. The method of study of bacterial flagellation recommended by Conn and Wolfe (5) was used.

The following tests and reactions were used for determining the biochemical characteristics of the isolated cultures: fermentation of lactose, dextrose, sucrose, salicin, dulcitol, and glycerol; formation of indol from 1 per cent tryptone broth; utilization of citrate as a sole source of carbon; methyl red and Voges-Proskauer reactions; reduction of nitrate to nitrite; hydrogen sulfide production from peptone-iron agar; gelatin liquefaction, and action on litmus milk.

As far as possible the media used for the biochemical characterization of the cultures were selected from the Manual of Methods for Pure Culture Study of Bacteria (18).

The formation of indol was determined by the Gore modification (18) of the Ehrlich-Böhme technique, after 3 days at 37° C.

The medium used for the study of citrate utilization was that given in Standard Methods for the Examination of Water and Sewage, 8th Edition, 1936 (1).

The methyl red and Voges-Proskauer reactions were determined according to Werkman's modification (21) and Barritt's modification (3) further modified by Vaughn, Mitchell, and Levine (20). The tubes treated for the Voges-Proskauer reaction by the Werkman modification were allowed to stand a half day or overnight to permit development of color.

The reduction of nitrate to nitrite was determined by the method given in the Manual of Methods for Pure Culture Study of Bacteria, Leaflet V, p. 8, 1939 (18).

The iron-peptone medium was a modification of the suggestion of Levine and co-workers (13, 14) and as recommended by Tittsler and Sandholzer (19).

Cultures that could not be identified on the first attempt were repurified, and biochemical studies were repeated.

The pH determinations were made with a Coleman glass electrode, pH electrometer, model 3D.

Identification of the cultures was made according to Bergey's Manual of Determinative Bacteriology, 5th Edition, 1939 (4).

#### PRESENTATION OF EXPERIMENTAL DATA

*Preliminary Studies.* Several preliminary observations were made, the first of which was to determine whether or not a presumptive medium with a 1:30,000 concentration of brilliant green had any appreciable inhibitory effect on certain strains of coliform organisms when a sterile ice cream mix, inoculated with these organisms, was planted into brilliant green-lactose-bile

broths containing dye concentrations of 1:30,000 and 1:75,188. The second preliminary study was made to determine whether a buffered brilliant green-bile broth gave a higher coliform count than an unbuffered broth when inoculated with a sample of a sherbet containing certain strains of coliform organisms.

Comparative studies were made with the same broths and violet red-bile

TABLE 1

*Comparative numbers of coliform group in factory-packed samples of frozen desserts*

Plant	Vanilla ice cream	Chocolate ice cream	Strawberry ice cream	Sherbet or ice	Cheerio
(a) Brilliant green-lactose-bile broth (most probable number per ml.)					
A	3.29	2.40	2.75	0.032	3.29
B	0.009	0	0	0	3.29
C	0.110	4.93	116.0	0.110	1.93
D	13.0	6.22	6.22	0	2.23
E	4.93	4.93	1,160.0	0.275	3.24
F	0	0.933	10.20	2.40	
G	3.29	3.29	3,490.0	0	33.40
H	15.0	0.493	4.93	0	17.0
I	474.0	197.0	150.0	0.101	116.0
J	0.214	19.7	0.087	2.31	2.31
K	1.01	0.792	1.71	0.168	6.22
L	0.073	0.275	0.032	0	0.78
M	6.22	0.217	9,180.0	0.020	79.20
N	0.792	0.053	13.0	0	17.1
(b) Violet red-bile agar (plate count per ml.)					
A	0	2	1	0	2
B	0	0	0	0	2
C	0	0	*	0	0
D	9	2	3	0	*
E	50	14	450	0	2
F	0	0	8	2	
G	0	0	26,900	0	100
H	*	*	*	0	*
I	130	50	85	0	3,600
J	1	10	0	0	*
K	1	0	2	0	0
L	0	0	0	0	0
M	20	0	6,850	0	40
N	0	0	1	0	10

\* Counts could not be made because of density of inoculum, formation of gas bubbles, or overgrowth by other organisms.

agar. Five separate determinations were made in each of two trials, where sterile vanilla ice cream mixes were inoculated with suspensions of separate strains of *Escherichia coli*, *E. freundii*, *Aerobacter aerogenes*, *A. cloacae*, and a mixture of *E. coli* and *A. aerogenes*.

No significant differences were found between the counts obtained on brilliant green-bile broths, containing the different dye concentrations, and on violet red-bile agar. In the case of the broths the percentage deviations above and below the mode, or most probable number, given by Halvorson

and Ziegler (10) were used to determine the lower and upper limits of variation. In the case of the violet red-bile agar, a deviation of 100 per cent above and below the average counts was assumed.

Comparative studies were made on buffered and unbuffered brilliant green-bile broths and on violet red-bile agar using a coliform-free orange sherbet inoculated with fairly heavy suspensions of separate strains of *E. coli*, *A. aerogenes*, and a mixture of the two species.

No significant differences were found in the three types of presumptive media. However, when 1.0 milliliter and 0.1 milliliter amounts of inoculum were used, there was an apparent inhibition of the coliform organisms, both in the buffered and unbuffered broths, but growth was unrestricted in higher

TABLE 2

*Comparison of most probable numbers of coliform group in sherbets and ices*  
Buffered and unbuffered brilliant green-lactose-bile broth

Plant	Type of sample	Most probable number	
		Buffered brilliant green-bile broth	Unbuffered brilliant green-bile broth
		<i>per ml.</i>	<i>per ml.</i>
A	Orange sherbet		0.032
B	Orange sherbet		0
C	Lime sherbet	0.020	0
D	Orange ice	0	0
E	Orange sherbet	0.329	0.231
F	Pineapple sherbet	2.400	2.400
G	Orange sherbet	0	0
H	Orange sherbet	0	0
I	Orange sherbet	0.128	0.078
J	Orange sherbet	2.310	2.310
K	Orange sherbet	0.125	0.103
L	Orange ice	0	0
M	Orange sherbet	0.020	0.020
N	Pineapple ice	0	0

dilutions. The inhibition may have been due to the low pH of the sherbet or ice.

*Numbers of the Coliform Group.* Table 1 shows (a) the most probable numbers of coliform bacteria in vanilla, chocolate, and strawberry ice cream, in sherbets (or ices), and in "Cheerio" samples received from 14 ice cream manufacturers. In general, reasonably comparable results were obtained on the two presumptive media used.

The only apparent significant differences in the results with the two media, as shown in table 1, are in the following samples: vanilla ice cream from Plant E, strawberry ice cream from Plants C, G, and N, and "Cheerios" from Plant I.

Table 1 shows also that by using an inoculum of 10 milliliters of the sample as the largest portion planted into the brilliant green-bile broth it was possible to detect the presence of smaller numbers of the coliform group

than could be shown by direct plating on violet red-bile agar. Twenty-five samples out of a total of 69, which showed no characteristic coliform colonies on violet red-bile agar gave positive tests in the brilliant green-bile medium, and of this number, 21 were confirmed.

The samples of sherbet from Plants A and B were planted into unbuffered brilliant green-bile broth (10 tubes inoculated in each dilution) and violet red-bile agar only. The samples of sherbets and ices from Plants C to N, inclusive, were planted into both buffered, and unbuffered, brilliant green-bile broths and in violet red-bile agar. The most probable numbers for the sherbets and ices, shown in table 1, were determined for the combined number of positive tubes in both the buffered and unbuffered liquid presumptive media. Table 2 shows a comparison of the most probable number of the coliform group, obtained in samples of sherbets and ices. No significant differences are shown in the counts obtained.

TABLE 3

*pH values of samples of sherbets and ices and of buffered and unbuffered brilliant green-lactose-bile broths + 10 ml. of sample*

Plant	Type of sample	pH of Sample	pH values	
			Buffered brilliant green-bile broth + 10 ml. sample	Unbuffered brilliant green-bile broth + 10 ml. sample
A	Orange sherbet			6.40
B	Orange sherbet	2.72		5.27
C	Lime sherbet	2.88	7.10	6.64
D	Orange ice	3.00	6.88	5.60
E	Orange sherbet	3.72	7.13	6.30
F	Pineapple sherbet	3.98	6.91	5.98
G	Orange sherbet	2.68	6.60	5.12
H	Orange sherbet	2.82	6.62	5.23
I	Orange sherbet	3.37	7.22	5.98
J	Orange sherbet	3.53	7.10	6.10
K	Orange sherbet	3.58	6.87	5.67
L	Orange ice	3.22	6.97	5.70
M	Orange sherbet	3.24	6.88	6.05
N	Pineapple ice	3.07	7.10	6.15

Table 3 shows that the increase in pH brought about by buffering ranged from 0.46 to 1.48, with the average increase in pH for 12 determinations being 1.07.

Table 4 gives the most probable numbers of coliform bacteria in 30 scoop samples of ice cream obtained from 30 different retail dealers. The only apparent significant differences in the results with the two presumptive media are in Samples 14 and 21.

It is demonstrated in table 4 also that by using brilliant green-bile broth as the presumptive medium it was possible to detect the presence of smaller numbers of the coliform group than could be shown by direct plating on violet red-bile agar. Six samples, out of a total of 30, which showed no

TABLE 4

*Comparative numbers of coliform group in scoop samples of ice cream*

Sample	Brilliant green-lactose-bile broth	Violet red-bile agar
	Most probable number	Plate count
	<i>per ml.</i>	<i>per ml.</i>
1	3.99	6
2	0.20	1
3	0.19	2
4	792.00	1,290
5	93.30	60
6	1.64	2
7	860.00	260
8	101.00	44
9	93.30	100
10	1.93	1
11	101,000.00	83,000
12	2.68	0
13	70,000.00	70,500
14	1.69	170
15	166.00	350
16	0.78	1
17	0	0
18	1.01	0
19	399.00	250
20	0.20	0
21	11,600.00	93,000
22	13,300.00	17,100
23	4.93	0
24	125.00	30
25	130.00	105
26	74.20	30
27	1.10	0
28	3.99	0
29	792.00	180
30	125.00	60

characteristic coliform colonies on violet red-bile agar gave positive tests in the brilliant green-bile medium, and of this number all were confirmed.

The relative numbers of the coliform group found in factory-packed samples and in scoop samples of ice cream are indicated in table 5. These results suggest the possibility of contamination during dispensing.

TABLE 5

*Relative numbers\* of coliform group in frozen desserts*

Type of sample	Per cent of samples showing			
	Zero coli-form count	Less than 10 per ml.	Less than 100 per ml.	More than 100 per ml.
Factory-packed (69 samples) .....	13.0	75.4	88.4	11.6
Scoop (30 samples) .....	3.0	46.7	56.7	43.3

\* Most probable numbers.

*Identity of Isolated Cultures.* The cultures isolated from the 69 samples of factory-packed ice cream and from 30 samples of scoop or dipper ice cream were identified by the usual methods. The results are presented in table 6. *Escherichia coli* was clearly a minor factor in these samples.

TABLE 6  
*Species of coliform group isolated from frozen desserts*

Organism	Factory-packed samples (227 cultures)	Scoop samples (89 cultures)
	<i>per cent</i>	<i>per cent</i>
<i>Escherichia coli</i>	5.3	1.1
<i>E. coli</i> var. <i>acidilactici</i>	1.3	1.1
<i>E. coli</i> var. <i>neapolitana</i>	3.1	5.6
<i>E. coli</i> var. <i>communior</i>	0.0	1.1
<i>Escherichia freundii</i>	27.3	28.1
<i>Aerobacter aerogenes</i>	23.8	18.0
<i>Aerobacter cloacae</i>	29.5	38.2
Non-coliform	9.7	6.8

Fifteen cultures, which were identified as non-coliform types, were isolated from positive tubes of brilliant green-bile broth which showed more than 10 per cent of gas after 48 hours' incubation at 37° C. These cultures were all single isolations (with one exception) and with the exception of five were Gram-negative rods. Two of the cultures appeared to be atypical *Aerobacter aerogenes* types on eosin-methylene blue agar while the others appeared to be non-coliform types. Microscopic examination showed one of the atypical *A. aerogenes* types to be a Gram-positive coccus, while three of the non-coliform types were Gram-positive cocci and one was a Gram-positive rod. It appears that the phenomenon of symbiosis or synergism may have been responsible for the presence of the gas in the presumptive tubes and also for the appearance of atypical *A. aerogenes* colonies on the eosin-methylene blue agar plates. Three cultures, identified as non-coliform, which resembled coliform types were isolated from violet red-bile agar plates. Symbiosis or synergism may have been involved again.

Studies were made of cultures isolated from tubes of brilliant green-bile broth that showed less than 10 per cent of gas after 48 hours incubation at 37° C. With one exception, only one culture was isolated from each of the ten tubes. These ten cultures represented ten different samples and with the exception of one culture were all Gram-negative rods. Six of the cultures were identified as coliform organisms.

The temporary appearance of eleven cultures with a ropy consistency on nutrient agar slants was noted. Five were identified, four as *Escherichia freundii* and the other a non-coliform type. This characteristic (ropiness) was not constant throughout the study of these cultures since it appeared on only one or two of the agar slants in the case of each culture.

The dependability of colony characteristics on eosin-methylene blue agar

plates as a basis for preliminary identification of coliform types is shown in table 7. In some cases, it was found that 48-hour incubation gave a better differentiation of colony types than 24-hour incubation.

TABLE 7  
Identity of coliform and non-coliform types found on eosin-methylene blue agar plates

Appearance of colony on E.M.B. plate	Per cent of colonies actually identified as				
	<i>E. coli</i>	<i>E. freundii</i>	<i>A. aerogenes</i>	<i>A. cloacae</i>	Non-coliform
Typical <i>E. coli</i> (73 cultures)	35.6	64.4			
Atypical <i>E. coli</i> (13 cultures)	30.8	61.5	7.7		
Typical <i>A. aerogenes</i> (70 cultures)		4.3	31.4	64.3	
Atypical <i>A. aerogenes</i> (120 cultures)		23.3	38.3	35.0	3.4
Non-coliform (40 cultures)		2.5	5.0	32.5	60.0

All coliform cultures isolated and identified were definitely Gram-negative. Motility studies showed the *Escherichia coli* and *Aerobacter cloacae* cultures to be predominantly motile while the *E. freundii* and *A. aerogenes* cultures consisted of both motile and non-motile types. The shape and size of the different cultures varied considerably. Of the 288 cultures of coli-

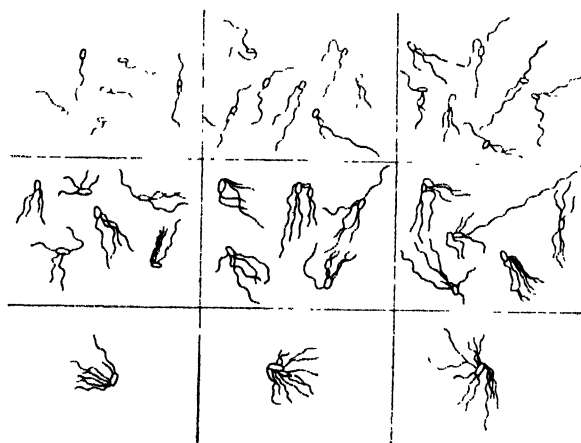


FIG. 1. Types of flagellation observed in cultures of coliform group studied.

form organisms 70.8 per cent showed peritrichic flagellation, 28.5 per cent atrichic, and 0.7 per cent monotrichic. The number and the length of the flagella and their positions on the organism varied considerably. Some cultures showed only one flagellum per organism while others showed 1 and 2, 1, 2, and 3, and so on. The largest number of flagella found on one bacterium was 12, this being a true peritrichic type. Flagella were found both



in the polar and lateral positions. (See figure 1, which was prepared from actual observation of stained cultures in this study.)

Fermentation studies showed that a number of coliform variants isolated in this study, had lost the ability to produce gas from some carbohydrates. A few variants were found which failed to produce both acid and gas from lactose. However, all other biochemical reactions and the morphological characteristics were normal and indicated that these variants were members of the coliform group. These data show that there was variability among certain strains of coliform organisms in their ability to ferment different carbohydrates.

Formation of indol, citrate utilization, gelatin liquefaction, and action on litmus milk for the cultures studied conformed closely to the reactions listed in Bergey's Manual of Determinative Bacteriology (4).

Usually an inverse correlation exists between the methyl red and the Voges-Proskauer tests. This study showed a greater degree of negative correlation between these two tests for Vaughn, Mitchell, and Levine's further modification of the Barritt modification than for Werkman's. The former method gave 33 positive Voges-Proskauer reactions that were negative according to Werkman's modification. The methyl red reactions were negative for these 33 tests. According to Bergey's Manual some strains of *Escherichia freundii* give both positive methyl red and positive Voges-Proskauer tests. Fourteen cultures of *E. freundii* which gave both positive methyl red and positive Voges-Proskauer tests were found in this study.

The ability of coliform organisms to reduce nitrates to nitrites is a property which is considered constant. However, nine coliform cultures which failed in this function were found. These cultures were typical in all other respects.

Although Bergey's Manual states that the majority of strains of *Escherichia freundii* produce hydrogen sulfide in peptone-iron agar, only 17 *E. freundii* cultures produced hydrogen sulfide in peptone-iron agar while 70 cultures failed to do so.

#### DISCUSSION

This study showed that many of the samples of frozen desserts had high coliform counts and that some contained the fecal coliform type, viz., *Escherichia coli*. The greater prevalence of the non-fecal *Aerobacter* species was definitely established, however, for this group of samples.

Since a dye concentration of 1:30,000 of brilliant green in brilliant green-lactose-bile broth had no appreciable inhibitory effect on certain strains of coliform organisms, it may be assumed that the presumptive medium with this higher dye concentration may be used for the detection of the coliform group in ice cream. Such a medium would have a greater selective action in inhibiting those Gram-positive organisms which are responsible for false presumptive tests.

Since no appreciable differences were shown in the coliform counts made in buffered and unbuffered presumptive media where the inocula were sherbets or ices, buffering does not appear to be necessary even for the more highly acid products.

It is possible to detect the presence of smaller numbers of coliform bacteria in the brilliant green-bile broths where a 10-milliliter or a 1-milliliter inoculum is used as the largest quantity planted than in the violet red-bile agar medium where a 1-milliliter inoculum is used. Another reason why the broth appears to be more satisfactory for determining low coliform counts is that it is more free from those defects which mask or interfere with reading the agar plates. Another fault of the solid medium is that certain non-coliform organisms may produce colonies that appear to be, or closely resemble, coliform colonies.

The "skips" which occurred in the case of three samples are characteristic of random sampling, and their presence in the dilution technique should not be considered unreasonable because such phenomena may occur where there are changes in the culture medium.

It seems reasonable to expect that factory-packed samples of ice cream should contain less than 10 coliform organisms per milliliter of sample. This figure appears to be fair since 75.4 per cent of the samples complied with it. It is realized, of course, that further work on the same subject is desirable and until that is done a coliform standard which will be applicable over a wide area cannot be set. Perhaps a coliform standard for scoop samples of ice cream should be established by sanitarians who are dealing with restaurants and other eating places.

#### SUMMARY AND CONCLUSIONS

1. Sixty-nine samples of factory-packed ice cream and 30 scoop or dipper samples of ice cream were studied to determine the numbers of the coliform group and the different types or species present therein.

2. A preliminary study failed to show that a dye concentration of 1:30,000 of brilliant green in brilliant green-lactose-bile broth had any appreciable inhibitory effect on certain strains of coliform organisms.

3. In general, no significant differences were found in the numbers of the coliform group present when comparisons were made between counts obtained in brilliant green-bile broth and on violet red-bile agar.

4. Comparisons were made between coliform counts obtained in buffered, and unbuffered, brilliant green-bile broths which had been inoculated with samples of sherbets or ices. The results were essentially the same in each case.

5. The range of coliform counts, in terms of most probable numbers, in the factory-packed samples was between 0 and 9, 180 per milliliter.

6. The range of the coliform counts (most probable numbers) in the scoop samples of ice cream was between 0 and 101,000 per milliliter.

7. Brilliant green-bile broth appeared to be a better presumptive medium than violet red-bile agar for the detection of the coliform group where the counts were quite low.

8. The cultures isolated from the factory-packed samples of ice cream were distributed as follows: *Escherichia coli*, 5.3 per cent; *E. coli* var. *acidilactici*, 1.3 per cent; *E. coli* var. *neapolitana*, 3.1 per cent; *E. coli* var. *communior*, 0.0 per cent; *E. freundii*, 27.3 per cent; *Aerobacter aerogenes*, 23.8 per cent and *A. cloacae*, 29.5 per cent. Non-coliform species isolated from these samples constituted 9.7 per cent of the total number.

9. The species and varieties isolated from the scoop samples were distributed as follows: *Escherichia coli*, 1.1 per cent; *E. coli* var. *acidilactici*, 1.1 per cent; *E. coli* var. *neapolitana*, 5.6 per cent; *E. coli* var. *communior*, 1.1 per cent; *E. freundii*, 28.1 per cent; *Aerobacter aerogenes*, 18.0 per cent, and *A. cloacae*, 38.2 per cent. Non-coliform species isolated from these samples constituted 6.8 per cent of the total number.

10. The results obtained in this study indicate that factory-packed samples of ice cream should contain less than 10 coliform organisms per milliliter of sample.

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# SEVENTY YEARS OF SELECTION FOR CONFORMATION IN DAIRY CATTLE\*

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For many years on the Island of Jersey in the English Channel there has been a system of inspection and classification for conformation of all registered Jersey cattle. Importations of Island cattle have frequently been brought to this country. The Island type Jersey has supplanted to a large extent, in the show-ring and leading breeders' herds, the larger, more rugged but rougher type of Jersey which was once popular here and was founded on the blood of Canadian Jersey families.

With the publication of the first volume of the herd book in 1866 by the Royal Jersey Agricultural and Horticultural Society of the Island there was inaugurated the official type classification of all Jerseys registered by the society (1). The written requirements for classification have been changed little since that time. Considerable emphasis apparently has been laid in the selection of breeding stock on the type classification of the cattle. A study of the results obtained through the long period of selection for improved conformation should be of interest to students of dairy cattle breeding.

Questions involved in the study are: What was the proportion of animals of superior conformation which produced the registered Island Jersey cattle during different periods? Did this proportion show any tendency to increase as a result of the program to improve conformation? What proportion of the matings were of animals of superior conformation? Did they show assortive mating? What proportion of offspring with excellent conformation resulted from these matings? Does this proportion show an increase as genes for poor conformation were removed from the population by the selection practised? Does there appear to have been any difference in the influence of the sire and the dam on the conformation of the offspring?

The requirements for registration are that when a qualified cow drops a calf, the birth must be attested within 24 hours by a member of the Agricultural Society. This calf must then be registered within eight days of birth. At this stage the calf is not given an entry number in the herd book and may be finally rejected. The first calf of a registered heifer, not yet qualified, is entitled to registration provided the sire and the dam's parents are quali-

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fied, and provided the dam is herself qualified within nine months of the birth of the calf.

Qualifications require a public examination of the animal by a panel of usually two or three judges. The qualities which must be possessed by the animals are left to the discretion of the judges and have undoubtedly changed somewhat with changing show ideals. The cattle are graded as "Commended" (C), or "Highly Commended" (HC), or are rejected. Animals of satisfactory conformation are then assigned a herd book number. A bull calf is inspected as a yearling and must be presented with its dam if she is on the Island. The merits of the dam are possibly considered in evaluating the bull. In that case the classification of the bulls should resemble that of the dam in more cases than it does the sire. In cases where it is necessary to show the bull without its dam it cannot be classed higher than "Commended" unless the dam has been a prize winner at an Island show.

Any animal that has been rejected may be offered for re-examination but is usually slaughtered. Heifers whose dams have not been registered may be accepted as foundation stock and the progeny, other than the first calf, may be registered. Ninety per cent of the dairy cattle on the Island in 1936 were registered Jerseys (1).

Animals which do not meet the requirements for at least "Commended" cattle may not be registered and are thus not recorded in the books of the Royal Jersey Agricultural and Horticultural Society. This provision considerably weakens the material from the standpoint of a study of breeding methods and results. The classification given at registration is retained throughout the life of the animal. Cases were observed in this study where "Commended" bulls had become champions of Island shows and had been extensively used.

In spite of there being no record of rejected calves an indication of the results of the classification program can be attained by comparing the proportions of "Commended" and "Highly Commended" progeny obtained. If the type requirements for these classifications changed much from period to period the proportions of "HC" and "C" calves would differ even if classification had been made of the same population. Any conclusions drawn must be predicated, therefore, on the assumption that classification standards did not change much or at least were not lowered.

#### METHOD OF TABULATION OF THE DATA

The data were obtained from the herd books of the Royal Jersey Agricultural and Horticultural Society. The classification of each animal was recorded and then the same information obtained for its dam and sire. Every animal listed in the registry books was tabulated, with a very few exceptions, from the first volume listed in table 1 and as far through the

TABLE 1

*The proportion of progeny from HC sires and from HC dams compared to the proportion of such parents*

Periods	HC sires	Progeny		HC dams	Progeny	
		Bulls	Heifers		Bulls	Heifers
	%	%	%	%	%	%
I 1866 to 1882 ♀ ♂	64.3	63.5	65.1	29.2	30.5	28.2
II 1890 to 1891 ♀ 1890 to 1893 ♂	82.1	89.0	75.3	76.0	84.6	67.4
III 1906 to 1907 ♀ 1906 to 1909 ♂	81.2	90.3	72.0	59.3	73.2	45.4
IV 1926 to 1927 ♀ 1922 to 1927 ♂	74.7	78.2	72.2	79.3	88.4	70.1
V 1935 ♀ 1930 to 1935 ♂	90.5	92.3	88.8	79.7	90.6	68.9
Summary	78.6 <sup>a, b</sup>	82.6 <sup>a, c</sup>	74.5 <sup>b, c</sup>	64.7 <sup>c, d</sup>	73.4 <sup>c, f</sup>	55.9 <sup>d, f</sup>

Sections I to V based on 1036 sires or dams and 518 bulls and 518 heifers.

Summary based on 5180 sires or dams and 2590 bulls and 2590 heifers.

Statistical significance (3) of the difference between selected proportions by

$$\sigma D\% = \sqrt{pq \left( \frac{1}{N_1} + \frac{1}{N_2} \right)} :$$

<sup>a</sup> HC sires and their male progeny, Summary Difference 4.0% ± 1.9.

<sup>b</sup> HC sires and their female progeny, Summary Difference 4.1% ± 2.0.

<sup>c</sup> HC dams and their male progeny, Summary Difference 8.7% ± 2.2.

<sup>d</sup> HC dams and their female offspring, Summary Difference 8.8% ± 2.3.

<sup>e</sup> Bull calves and heifers from HC sires, Difference 8.1% ± 1.7.

<sup>f</sup> Bull calves and heifers from HC dams, Difference 17.5% ± 1.3.

last volume listed as it was necessary to go in order to obtain 518 male and the same number of female progeny in each group. Since five such groups of data were obtained for five periods since 1866 the total number of offspring tabulated in this study was 5180. The classification of dam and sire was obtained wherever listed. Each mating which resulted in a registered offspring was recorded as a separate entity. The actual number of parents would not be double that of the progeny since popular and long-lived bulls sired many calves and thus tended to dominate the results.

## RESULTS

I. *The type classification of parents of Island Jersey cattle. Proportion of "Commended" and "Highly Commended" parents.* Since the numbers of cattle registered were few in the first years of registration, a period of 16 years had to be covered to secure the necessary number of progeny for period I (table 1). During this period slightly less than two-thirds of the sires were classified as "Highly Commended" but only 29.3 per cent of the dams. The proportion of "HC" parents in the 1890-3 period had increased to 82.1 per cent for the sires and 76 per cent for the dams, showing a strong tendency to breed to animals of better conformation unless



the classification standards had been dropped. In the 1906-9 period the percentage of "Highly Commended" dams dropped to 59.3 per cent, while the proportion of such sires was maintained near the previous level. Possibly the requirements for "HC" classification of dams had become more stringent or breeders had decreased their emphasis on the use of better type dams. The latter condition would result if there were increased sale of stock for exportation. A further increase in use of excellent type bulls occurred in the 1930-5 period. The surprising proportion of 90.5 per cent of the sires of calves registered during that period were of "Highly Commended" conformation.

*Proportion of different matings producing registered progeny.* There are four possible matings between "Commended" and "Highly Commended" cattle. They are "C" sires  $\times$  "HC" dams, "HC" sires  $\times$  "C" dams, "C" sires  $\times$  "C" dams, and "HC" sires  $\times$  "HC" dams (table 2). In the earliest period studied, 27.1 per cent of the 1036 matings were of "Commended" sires to "Commended" cows. Matings of "HC" cows and "C" bulls produced only 8.6 per cent of the registered calves. The mating of "HC" bulls to "C" dams produced 43.6 per cent of the registered calves while one-fifth were from bulls and cows both of which were "Highly Commended" (table 2).

In the 1890-3 period the proportion of "C"  $\times$  "C" matings had declined to 4.9 per cent. Matings of "Commended" bulls to "Highly Commended" cows had, however, increased considerably. Registered calves from "HC" sires  $\times$  "C" dams had also declined to less than half the proportion found in the first period due especially to a decrease in the number of registered bull calves from this mating. "HC"  $\times$  "HC" matings produced over three-quarters of the registered bull calves and half of the heifers.

During the 1906-9 period either the stringency of selection for conformation declined or the classification standards were raised for the number of registered calves produced by "C"  $\times$  "C" matings had increased considerably (table 2). Only eight per cent of the matings were of "C" sires  $\times$  "HC" dams. The mating of "HC" sires  $\times$  "C" dams produced almost 30 per cent of the registered calves. "HC"  $\times$  "HC" matings had consequently declined to about half.

The 1922-7 period showed a considerable increase in matings of "C" sires  $\times$  "HC" dams and a decline in the proportion of "HC" sire  $\times$  "C" dam matings. "C"  $\times$  "C" matings had declined to the 1890-3 figure. "HC"  $\times$  "HC" matings producing registered bull calves did not change much but heifers from the same matings increased 13 per cent.

In the latest period, 1930 to 1935, almost three-quarters of the matings were of "HC"  $\times$  "HC" parents. Very few were "C"  $\times$  "C" matings, 17.5 per cent "HC"  $\times$  "C" and 6.7 per cent "C"  $\times$  "HC."

From table 2 may be calculated the amount of assortive mating which

TABLE 2

*Proportion and number of the four matings of Island Jersey cattle producing registered progeny*

Matings Sires × Dams	Percentage of progeny			Number of progeny		
	Both sexes	Bulls	Heifers	Both sexes	Bulls	Heifers
Period I: 1866-1882						
C × C	27.1	28.8	25.5	281	149	132
HC × C	43.6	40.7	46.5	452	211	241
C × HC	8.6	7.7	9.5	89	40	49
HC × HC	20.7	22.8	18.5	214	118	96
All matings	100.0	100.0	100.0	1036	518	518
Period II: 1890 to 1891-3						
C × C	4.9	1.5	8.3	51	8	43
HC × C	19.1	13.9	24.3	198	72	126
C × HC	12.9	9.5	16.4	134	49	85
HC × HC	63.0	75.1	51.0	653	389	264
All matings	100.0	100.0	100.0	1036	518	518
Period III: 1906 to 1907-9						
C × C	10.8	2.9	18.7	112	15	97
HC × C	29.9	23.9	35.9	310	124	186
C × HC	8.0	6.8	9.3	83	35	48
HC × HC	51.3	66.4	36.1	531	344	187
All matings	100.0	100.0	100.0	1036	518	518
Period IV: 1922-6 to 1927						
C × C	4.8	1.7	7.9	50	9	41
HC × C	15.9	9.9	22.0	165	51	114
C × HC	20.5	20.1	20.8	212	104	108
HC × HC	58.8	68.3	49.2	609	354	255
All matings	100.0	100.0	100.0	1036	518	518
Period V: 1930-5 to 1935						
C × C	2.8	1.5	4.1	29	8	21
HC × C	17.5	7.9	27.0	181	41	140
C × HC	6.7	6.2	7.1	69	32	37
HC × HC	73.1	84.4	61.8	757	437	320
All matings	100.0	100.0	100.0	1036	518	518
Summary						
C × C	10.1	7.3	12.9	523	189	334
HC × C	25.2	19.3	31.2	1306	499	807
C × HC	11.3	10.0	12.6	587	260	327
HC × HC	53.4	63.4	43.3	2764	1642	1122
All matings	100.0	100.0	100.0	5180	2590	2590

occurred during these periods. If much attention was paid to the type classification of these cattle the "Highly Commended" cattle should usually have been mated to similar animals. Breeders with "Commended" cattle,

especially bulls, would be expected to be less particular about whether their animals were mated with "C" or "HC" animals. Owners of "Com-mended" cows might also be anxious, however, to improve the type of the offspring by mating to "HC" bulls. Any such matings, although tending to improve the conformation of the breed, reduce the amount of assortive mating. The coefficient of association used for calculating assortive mating (4) shows whether animals with similar type classification tended to be mated with similar or with dissimilar mates. This coefficient of association is rather erratic and not very dependable except for distinguishing between rather high proportions of assortive mating and none.

The amount of assortive mating varied considerably during different periods in this population (table 3). In only the 1906-9 period was there a significant amount of assortive mating in the matings which produced registered heifers. In the other periods the amount of assortive mating was not statistically significant according to the chi square.

TABLE 3

*The proportion of assortive mating among the parents of registered Island Jersey cattle*

Period	All matings		Matings			
			Producing bulls		Producing heifers	
1866-82	.20 <sup>a</sup>	7.5 <sup>b</sup>	.35 <sup>a</sup>	12.2 <sup>b</sup>	.04 <sup>a</sup>	.12 <sup>b</sup>
1890-93	.11	1.5	-.06	.10	.03	.08
1906-09	.40	28.8	.09	.28	.34	12.2
1922-27	-.07	.59	-.25	1.85	-.08	.58
1930-35	.28	5.8	.45	5.63	.13	.80
Summary	.31	8.6	.41	67.1	.17	15.07

<sup>a</sup> Yule's (4) coefficient of association for two-fold table

$$q = \frac{ad - bc}{ad + bc}$$

<sup>b</sup> Chi square for two-fold table (3)

$$\chi^2 = \frac{(ad - bc)^2 (a + b + c + d)}{(a + b)(c + d)(a + c)(b + d)}$$

A chi square value of 3.841 or more is considered significant.

In the matings which produced the registered bull calves the amount of assortive mating was highly significant in two widely separated periods, 1866-82 and 1930-5. In the matings which produced bull calves from 1922-7 there was some tendency to mate the parents to animals of the other type classification. The figure for assortive mating, although high, does not appear to be statistically significant.

The nature of the dairy business on the Island was such that the management of herds from which most of the bulls were registered might well have followed practices different from those where only heifers were registered. Bulls were kept by a few breeders with the larger herds. Cows were brought from the neighboring small herds to be bred. Figures on assortive mating

based on an equal number of bulls and heifers are of doubtful significance as in the column of "All Matings," table 3.

II. *The progeny from Commended and Highly Commended parents. The proportion of Highly Commended offspring.* In the 1866-88 period tabulated, only 24.1 per cent of the bull calves and 18.5 per cent of the heifers registered were classified as "Highly Commended" (table 4). This proportion jumps, however, in the 1890-3 period to about two-thirds for the male and 71.2 per cent for the female progeny. The 1906-9 period showed a decline in the proportion of "Highly Commended" progeny to close to 50 per cent for both sexes. This corresponded to a considerable decline in the proportion of "Highly Commended" dams in the group but occurred in spite of 81.2 per cent of the sires being "Highly Commended." Recovery was good in the 1922-7 period when practically 70 per cent of both bull calves and heifers registered during that period were classed as "Highly Commended." The remaining 30 per cent were, of course, classified as "Commended."

There was no consistent increase in the proportion of "Highly Commended" progeny produced, when compared to the proportion of similarly classified parents (table 4). These figures were obtained by dividing the number of "HC" progeny in each period by the number of "HC" sires plus the "HC" dams. Since two parents are required to produce one offspring the proportion of "HC" progeny to "HC" parents could not rise above 50 per cent even if all parents produced only "HC" progeny. The proportion for the first period is 22.8 per cent, rises to 43.2 per cent for the second period, is 36.7 per cent for the third, 45.3 for the fourth, and 36.4 per cent for the fifth period. This is consistent with the genetic view of the mass selection (2). Mass selection of epistatic factors results in rapid improvement during only the first few generations it is practiced. The improvement can then only be maintained through stringent culling. If the vigilance of selection is reduced the population tends to return to the original condition. Only through inbreeding can the homozygosity of the population be appreciably increased under practical conditions.

The increase between the first and second periods is highly significant. It indicates either a lowering of standards or the elimination of considerable inferior germ plasm. In later periods the proportion of "HC" progeny fluctuated considerably.

Examining the "HC" calves which were produced by "HC" sires it is seen that only in the 1866-82 period is the proportion of heifers greater than that of the bull calves (table 4). There was evidently a stronger tendency to register bull calves than heifers from "HC" sires during the second and third periods by 13.7 and 18.3 per cent. This tendency practically disappeared in the last two periods for bull calves from "HC" sires were only 5 and 3.5 per cent more numerous than heifers. Of course, in this study the numbers of bull calves and heifers in each group were kept equal at 518.

TABLE 4  
*Proportion of Highly Commended progeny from the four matings of registered Island Jersey cattle*

Sires × Dams	Percentage of HC progeny*			Proportion HC parents† to HC progeny
	Both sexes	Bulls	Heifers	
Period I: 1866 to 1882				
All matings	21.3	24.1	18.5	22.8
C × C	15.3	13.4	17.4	
HC × C	18.6	23.2	14.5	
C × HC	27.0	25.0	28.6	
HC × HC	32.7	40.0	25.0	
Period II: 1890 to 1891-3				
All matings	68.2	65.3	71.2	43.2
C × C	66.7	50.0	69.8	
HC × C	59.1	59.7	58.7	
C × HC	71.6	61.2	77.7	
HC × HC	70.4	67.1	75.4	
Period III: 1906 to 1907-9				
All matings	51.0	52.5	49.4	36.7
C × C	32.1	20.0	34.0	
HC × C	39.4	39.5	39.3	
C × HC	51.8	42.9	58.3	
HC × HC	61.6	59.6	65.2	
Period IV: 1922-6 to 1927				
All matings	69.7	69.5	69.9	45.3
C × C	62.0	66.7	61.0	
HC × C	59.4	52.0	63.2	
C × HC	71.2	65.4	76.9	
HC × HC	72.6	73.5	71.4	
Period V: 1930-5 to 1935				
All matings	62.1	63.7	60.4	36.4
C × C	48.3	50.0	47.6	
HC × C	49.2	51.2	48.6	
C × HC	63.8	81.3	48.7	
HC × HC	65.5	63.8	67.8	
Summary—Five Periods				
All matings	54.5	55.0	54.0	38.1
C × C	30.2 <sup>a</sup>	19.6	36.2	
HC × C	39.0 <sup>a, c</sup>	37.7	39.9	
C × HC	61.0 <sup>b, c</sup>	57.3	*63.9	
HC × HC	64.9 <sup>b</sup>	64.0	66.3	
Summary—Last Four Periods				
All matings	42.7	62.7	62.7	40.3
C × C	47.5	42.5	48.5	
HC × C	49.9	48.3	50.7	
C × HC	67.1	63.2	70.1	
HC × HC	67.6	65.9	70.2	

\* The numbers of registered progeny are the same as the numbers given in table 2.  
 HC progeny

† HC sires + HC dams

Statistical significance of the difference between selected proportions:

<sup>a</sup> Both sexes from C × C and HC × C dams matings, Summary Diff. = 9% ± 2.5.

<sup>b</sup> Both sexes from C sires × HC and HC × HC matings, Summary Diff. = 3.9% ± 2.2.

<sup>c</sup> Both sexes from HC × C dams and C sires × HC matings, Summary Diff. = 22.0% ± 2.5.

Calves registered from "HC" dams show a more marked numerical superiority of bull calves over heifers. During the second, third, fourth and fifth periods bull calves exceeded heifers by 17.2, 27.8, 18.3 and 21.7 per cent. These figures are highly significant and seem to indicate either that there was a stronger tendency to register bull calves from "HC" dams than from "HC" sires or that bull calves being shown for classification with their "HC" dams had a better chance of being accepted than heifers which were shown alone. The latter does not appear to be the true explanation, however, because of data presented later.

*The proportion of "HC" calves from different matings.* A tabulation of the results secured in the proportion of offspring "Highly Commended" from the four matings of Island Jersey cattle shows significant differences in certain instances and a surprising lack of difference in others.

Thirty per cent of the progeny of "Commended"  $\times$  "Commended" cattle were classed as highly commended. Of this proportion 19.6 per cent were bull calves and 26.2 per cent heifers. The variation in highly commended calves registered from this mating is from 15.3 per cent in the first period to two-thirds in the second. Eliminating the first period when a very low proportion of the offspring were being classed as "HC" an average of 47.5 per cent were given this classification in the last four periods (table 4). This disparity between the average proportion of "HC" progeny for all five periods and for the last four is due to the large proportion of the total "HC" bull calves from this mating which occurred in the earliest period. Eliminating the first period, the proportion of "HC" calves is 48.6 per cent for the heifers and 42.6 per cent for the bull calves.

*The mating of "Highly Commended" bulls to "Commended" cows* resulted in a total of 39 per cent "HC" progeny. This varied from 18.6 per cent in the first to 59.4 per cent in the third period. Eliminating the first period the proportion of "HC" calves from this mating was practically 50 per cent.

Using "Highly Commended" in place of "Commended" bulls did not change substantially the proportion of "HC" offspring resulting. In fact, when the last four periods are considered, practically the same proportion of "HC" calves resulted from "HC"  $\times$  "C" matings as from "C"  $\times$  "C" matings, 47.3 and 49.9 per cent, respectively. The average of all five periods gives, however, the "HC"  $\times$  "C" mating an advantage of 9 per cent, which is 3.6 times the standard error.

One might hastily conclude that since bull calves are shown with the dams that these "Commended" dams had influenced the judges' decisions to a considerable extent, thus producing this similarity. Instead, however, of the proportion of "HC" bull calves being low from the "HC" bull  $\times$  "C" cow mating, the proportion is higher in four out of the five groups of data than is the percentage of "HC" heifers. The averages for the five periods

are very close, 37.7 and 39.9 per cent, as are the averages for the last four groups, 48.3 and 30.4 per cent, respectively, for bulls and heifers. It does not seem reasonable that the classification of the dams would affect that of the heifers for the latter are not shown with them. Is it possible that the influence of the sire on the conformation, taken as a whole, of his offspring is less than that of the dam? Or has the selection practiced resulted in this situation?

The proportion of "HC" calves registered from the mating of "*Commended*" sires and "*Highly Commended*" dams was 61 per cent. The variation was from 27 per cent in the first to 71.6 in the second period. Eliminating the first period, 67.1 per cent of the progeny was classed as "HC."

The remarkable increase in proportion of "HC" progeny from this mating (61 per cent) compared with that obtained with the "C"  $\times$  "C" (30.2 per cent) and "HC" sires  $\times$  "C" dams (39 per cent) matings is worthy of note.

This difference is highly significant, being based on 587 progeny from "C" male  $\times$  "HC" female matings, 1306 progeny from "HC" male  $\times$  "C" female matings, and 523 from "C"  $\times$  "C" matings (table 4). The difference between the proportions of "HC" progeny from "HC" sires  $\times$  "C" dams and "C" sires  $\times$  "HC" dams is 6.8 times the standard error. If this high proportion of "HC" offspring from "C" sires  $\times$  "HC" dams were due to the bull calves being shown with their dams, then a higher proportion of male progeny than of female should be "Highly Commended" in this group of data. This, however, is not the case. The average for five periods is 63.9 per cent of the heifers "Highly Commended" and 57.3 per cent of the bull calves. The proportion of "HC" bull calves is lower in four of the groups of data for in only the 1930 to 1935 period, when the remarkable proportion of 81.3 per cent "HC" bulls was secured, does the proportion of "HC" males exceed that of the "HC" females. The average of the last four groups of data shows 63.2 per cent of the males "Highly Commended" as compared to 70.1 per cent of the heifers.

The mating of "*Highly Commended*" bulls to "*Highly Commended*" cows resulted in about 65 per cent "HC" calves (table 4). The variation in different periods is from 32.7 in the first to 72.6 in the third. Eliminating the first period, 67.6 per cent of the calves were "Highly Commended." The average for the five groups is not significantly different at 64.9 per cent from that secured when "Commended" bulls were mated to "Highly Commended" cows (61 per cent), being only 1.86 times the standard error. These figures are based on 2764 and 587 offspring, respectively. Almost the same proportion of bull calves was classed as "HC" (64 per cent) as of heifers (66.3 per cent).

*Progeny from "HC" dams compared with those from "C" dams.* The

results secured in classification of progeny seemed to be little influenced by the classification of the sires. Progeny from the two matings, "HC" × "HC" and "C" sires × "HC" dams, were then combined and the propor-

TABLE 5

*Proportion of HC progeny when the dams are Commended (C × C and HC sires × C dams combined) compared with the proportion when the dams are highly Commended (C sires × HC dams combined with HC × HC matings)*

HC progeny from Commended dams				HC progeny from Highly Commended dams		
Both sexes	Bulls	Heifers		Both sexes	Bulls	Heifers
Period I: 1866-1882						
16.3	19.2	15.5	Percentage	31.0	35.4	26.2
733	360	373	Total No. progeny	303	158	145
Period II: 1890 to 1891-3						
60.6 <sup>a, b</sup>	58.7	61.6	Percentage	70.6 <sup>a</sup>	66.4	75.9
249	80	169	Total No. progeny	787	438	349
Period III: 1906 to 1907-9						
37.4 <sup>b</sup>	37.4	37.5	Percentage	60.3	58.0	63.8
422	139	283	Total No. progeny	614	379	235
Period IV: 1922-6 to 1927						
60	53.3	62.6	Percentage	72.2	71.6	73.0
215	60	155	Total No. progeny	821	458	363
Period V: 1930-5 to 1935						
49.1	51.0	48.5	Percentage	65.4	65.0	65.8
210	49	161	Total No. progeny	826	469	357
Summary						
36.5 <sup>c</sup>	32.7 <sup>d</sup>	38.8 <sup>d</sup>	Percentage	64.3 <sup>c</sup>	63.1 <sup>e</sup>	65.8 <sup>e</sup>
1829	688	1141	Total No. progeny	3351	1902	1449

Statistical significance of the difference between selected proportions by

$$\sigma D\% = \sqrt{pq \left( \frac{1}{N_1} + \frac{1}{N_2} \right)} :$$

<sup>a</sup> Both sexes from C and HC dams: Period II. Difference = 10% ± 3.4.

<sup>b</sup> Both sexes from C dams: Periods II and III. Difference = 23.1% ± 3.9.

<sup>c</sup> Both sexes from C and HC dams: Summary Difference = 27.8% ± 1.5.

<sup>d</sup> Bulls and heifers from C dams: Summary Difference = 6.1% ± 2.3.

<sup>e</sup> Bulls and heifers from HC dams: Summary Difference = 2.7% ± 1.7.



tion of "HC" and "C" calves compared with the results secured when the other two matings, "HC" sires  $\times$  "C" dams and "C"  $\times$  "C," were combined (table 5).

The average results from the two types of matings based on 1829 calves from "Commended" dams and 3351 from "Highly Commended" dams show about one-third of the calves "HC" in the former and almost two-thirds in the latter case. The difference is extremely significant statistically, being 19.2 times the standard error. When the 1866-82 period, in which the proportion of "HC" offspring was low, is eliminated one-half of the offspring from "Commended" dams were "HC" while two-thirds of those from "HC" dams were classed as "HC." The differences in proportion of "HC" offspring obtained in the two groups of data between bull calves and heifers, although 6.1 per cent where the dams are "Commended" are in neither case statistically significant. It can be concluded, therefore, that although the bull calves were shown with dam they received substantially the same unbiased judgment as did the heifers.

The proportion of "HC" progeny secured was less during the 1866-82 period with both types of groupings, i.e., 16.3 per cent when the dams were only "Commended" and 31 per cent with "Highly Commended" dams. Results for the 1890-3 period, to 1891-3, were only 10 per cent apart with 60.6 per cent "HC" progeny from "Commended" dams and 70.6 from "Highly Commended" dams. During the 1906-9 period the proportion of "HC" progeny from both matings dropped somewhat but the difference in "HC" results when the dams are "HC" is still 22.9 per cent greater than when the dams are only "Commended." In the 1922-7 period the difference is 12.2 per cent, and 16.3 per cent in the 1930-5 period.

It is possible that the explanation for the high per cent "HC" calves from the "C"  $\times$  "HC" mating lies in the fact that bull calves shown without dam cannot be classed better than "Commended." Several cases were encountered in this study of "Commended" bulls which had become champions of Island shows and were extensively used. These bulls possessing "HC" conformation when mated to "HC" cows raised the proportion of "HC" progeny above that to be expected from the mating. To account for the low proportion of "HC" calves from the "HC" sire  $\times$  "C" dam mating on this basis one would have to conclude that the "HC" sires which were throwing a low proportion of "HC" calves tended to be mated to "C" cows.

#### SUMMARY

A study is reported of 5180 "Commended" and "Highly Commended" Island Jersey cattle and their parents. The data were tabulated in five period groups from 1866, including practically all calves registered during each period.

It was found that the proportion of the sires of Island Jersey cattle which were classified as "Highly Commended" increased from 64 per cent in 1866-82 to 82 per cent in 1890-3, declined somewhat in the 1922-7 period but rose to the surprising proportion of 90 per cent in 1930-5. Only 29 per cent of the dams were "HC" in 1866-83 but over  $\frac{2}{3}$  were so classified in three of the following periods. There appeared to be a greater tendency to select "HC" bulls than heifers from "HC" sires and dams.

The mating of "Commended" sires to "Commended" dams declined from 27 per cent of the total matings in 1866-82 to 2.8 per cent in 1930-5. "HC" males  $\times$  "C" dams declined from 43.6 per cent to 17.5 per cent while "HC"  $\times$  "HC" matings increased from 21 per cent to 73 per cent in the same periods. There appeared to be considerable assortive mating among the parents of registered bull calves in the 1866-82 and 1930-5 periods. Only in the 1906-9 period was assortive mating appreciable among the parents of heifer calves. The proportion of "HC" progeny to "HC" parents shows a considerable increase, from 23 per cent to 43 per cent between the first and second periods and thereafter considerable fluctuation but no consistent increase. The assumption is that no increase in homozygosity for the genes which influence "HC" conformation occurred after the 1890-3 period.

When the dams were only "Commended" 36.5 per cent of the calves received the rating of "HC" whereas when the dams were "HC," 64.3 per cent of the progeny received this rating. When "HC" sires were mated to "Commended" dams there were only 9 per cent more "HC" progeny. The same change when the dams were "HC" caused an increase of only 3.9 per cent in the proportion of "HC" progeny. Whether this was due to a greater genetic influence of the dam on her offspring or was a result of the selection practiced was undetermined. It could be due to the "Commended" classification of some better type bulls because of being shown without dam and to the genetically poorer "HC" sires being generally mated to "C" dams.

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## COMPARATIVE PALATABILITY OF SOME CEREAL PASTURES\*

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Cereal crops are used extensively as pasture crops in Kansas. Wheat grown for grain is quite often pastured in late fall and early spring without decreasing grain yield if judiciously done (3). Such practices occur in areas where large acreages of wheat are grown and relatively few live-stock are available, thereby resulting in large numbers of acres per animal unit. In the southwestern section of the state where winter wheat is the primary crop and winters are often favorable to excellent wheat growth, it has been profitable to utilize wheat pasture by shipping in cattle and sheep for pasture, usually on a contract basis. Wheat is seldom planted as a supplemental pasture crop only, although several acres are sometimes fenced off exclusively for pasture purposes. Winter barley comes on earlier and makes more winter growth than does wheat. For that reason it is recommended in some states as a special pasture crop. In much of Kansas the hazard of winter killing makes barley less dependable than wheat or rye for pasture. Rye is more universally planted for pasture purposes only than any of the cereals in Kansas. Its rank growth compared with the other small grains, and its ability to withstand severe winters makes rye a dependable, heavy yielding supplementary pasture crop. Common rye is used by most dairymen as a means of extending the pasture season through late fall and early spring grazing when other pastures are not available. It is also used when the pasture program consists primarily of a sequence of special pasture crops, either with or without native or tame pasture. Improved varieties of rye have been developed in recent years. Among the most promising varieties is Balbo, which has developed in Tennessee (2). It appears to be winter hardy in Kansas and produces more pasture than common rye. Another advantage is that it grows more upright during the grazing period than does common rye or wheat.

Differences of opinion among stockmen regarding the relative palatability of these cereals as pasture crops prompted this investigation. It is realized that palatability may not be of paramount importance in pasture crops, particularly when used alone, because cows will often do well on relatively unpalatable pasture crops if nothing else is available. Palatability would seem worthy of some consideration along with other factors in the selection of pasture crops, however, especially for high producing dairy cows

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when maximum food intake is important. Just how much influence palatability has on the amount of pasture consumed by dairy cows is unknown.

In this study a five-acre field was planted in four strips to Reno barley, Turkey wheat, common rye, and Balbo rye. Seeding was done on October 9, 1939, at the rate of two bushels per acre with a small grain drill. Six cows were used, two each of the Holstein, Ayrshire and Jersey breeds. The cows were being fed grain and silage, besides hay at night. It was thought that well fed cows would more truly reflect differences in palatability of the pastures than would cows which were so hungry that they would eat any kind of pasture. When turned out, however, all these cows ate pasture with relish. Cows varying in production were purposely selected to determine whether plane of production would be a factor in palatability or grazing time. The production plane varied from two dry cows to a cow giving more than 70 pounds of fat per month.

As a matter of convenience in gathering data, the cows were turned into the pasture at about 1:30 P.M. The cows received no pasture during the six-day period except in the afternoon of each day. Observations were taken at one minute intervals by recording the length of time spent in grazing on each strip by each cow until all cows had ceased grazing. The observations were taken on six consecutive days (March 19-24, 1940) and each day the cows were driven to different strips at the beginning of the grazing period. All the plots were ideal for pasture purposes, the plants averaging from four to six inches high with good stands.

TABLE 1

*Palatability of cereal pastures as measured in grazing time by dairy cows  
(Mar. 19-24, 1940)*

Cow No.	Lbs. fat daily	Grazing time									
		Balbo rye		Common rye		Winter wheat		Winter barley		Total	
		Ave. No. min.	Per cent	Ave. No. min.	Per cent	Ave. No. min.	Per cent	Ave. No. min.	Per cent	Ave. No. min.	Per cent
133	1.4	51	57	32	35	7	8	0	0	90	100
144	1.1	44	49	24	27	20	22	2	2	90	100
272	Dry	47	60	10	13	20	25	2	2	79	100
267	2.4	47	60	22	28	6	8	3	4	78	100
322A	Dry	42	47	19	21	22	25	6	7	89	100
319A	0.2	39	44	15	17	15	17	20	22	89	100
Ave.		45	52	21	24	15	18	5	6	86	100

Summary of the results (Table 1) showed that the average time spent in grazing the four different cereals was: Balbo rye—45 minutes, or 52 per cent of the total time; common rye—21 minutes, or 24 per cent; wheat—15 minutes, or 18 per cent; and barley—5 minutes, or 6 per cent. Without

exception, the cows showed a pronounced preference for Balbo rye, but the cows varied some in their relative preference for the different crops. A notable difference was the time spent in grazing barley by cow No. 319A, while all the other cows practically refused to stay on the barley plot even when driven on to it. Some variations existed with individual cows from day to day during the six-day period, but there were few daily exceptions in the order of preference for the different pasture plots.

The cows grazed for an average of approximately an hour and a half. The time spent in grazing was quite uniform, regardless of plane of butterfat production, the shortest average grazing times being recorded for the heaviest producing cow and a dry cow. These results are in agreement with the report of Fuller (1) who found that cows on winter rations spent approximately the same time eating, regardless of production. He stated also that about one and one-half hours were required for the cows to eat their grain, silage and hay. The short time required for cows to get their fill of pasture is of interest in both pasturing methods and cattle management.

These results show that cows have such a distinct dislike for barley that palatability would be an important factor in considering its value in comparison with other cereals as a pasture crop. Gross observations of herd cows over longer periods of time substantiate these conclusions. The pronounced preferences for Balbo rye by all the cows is of particular importance considering its heavier yield and more desirable growth habits. Although the preference shown for common rye over wheat was not so striking, the rank in palatability again agrees with rank in yield of pasture. It might be well to emphasize, however, that all plots represented good pasture conditions, and although Balbo rye was tallest, that factor was not the measure of palatability because the barley ranked second in height and appeared to be the most uniformly good pasture of the group.

#### SUMMARY AND CONCLUSIONS

Comparative palatability of the following cereal pastures was measured: Balbo rye, common rye, Turkey wheat and Reno barley. A five-acre field was divided into four strips and planted to these small grain crops in the fall. The time spent by six dairy cows of varying production in grazing each crop was recorded. The trial was conducted in the spring under ideal pasturage conditions.

The cows spent an average of 45 minutes, or 52 per cent of the grazing time, on Balbo rye; 21 minutes, or 24 per cent on common rye; 15 minutes, or 18 per cent, on wheat; and 5 minutes, or 6 per cent, on barley. The preference for Balbo rye and the dislike for barley was uniform for all the cows but the relative time spent on each of the cereals varied with individual cows.

The cows grazed an average of approximately an hour and a half to get their fill. The time spent in grazing was quite uniform, regardless of plane of production.

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# THE NUTRITIVE VALUE OF ALFALFA HAY. I. CYSTINE AS A SUPPLEMENT TO AN ALL ALFALFA HAY RATION FOR MILK PRODUCTION\*

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The experimental work reported in this paper is one phase of an extensive project initiated for the purpose of investigating the nutritive value and methods of feeding alfalfa hay to dairy cattle for efficient milk production. Since the trend in the feeding of dairy cattle is toward the greater use of home-grown rations it becomes imperative to have additional knowledge concerning various nutritional factors affecting milk production. The results reported in this paper are of further interest because of the absence in the literature of data on the effect of feeding cystine to lactating dairy cows.

In a previous report (11) it was indicated that alfalfa hay grown in the vicinity of East Lansing, Michigan, and fed as a sole ration to milking cows was deficient in a factor or factors essential for efficient milk production. The addition of isocaloric amounts of either corn or beet pulp in place of some of the alfalfa resulted in a marked increase in milk production. More recent work (13) has shown that the addition of either corn starch or glucose to an all alfalfa ration was much less effective than the addition of corn or beet pulp. The literature pertaining to the nutritive value of alfalfa hay has been summarized by Graves and co-workers (7) and Huffman (12).

The earlier work on the amino acid content of alfalfa protein has been reviewed by Mitchell and Hamilton (19). Chibnall (1) has summarized the amino acid content of alfalfa protein which was reported in the literature by Lugg (15, 16) and Tristram (22) as follows (expressed as percentage of protein) :

Arginine .....	8.0	Lysine .....	6.2
Aspartic acid .....	8.8	Methionine .....	2.3
Cystine .....	1.9	Tyrosine .....	6.1
Glutamic acid .....	11.4	Tryptophane .....	2.4
Histidine .....	1.5		

The possibility of a cystine deficiency in alfalfa protein has been indicated by the work of Haag (8) with growing rats. Rats receiving alfalfa leaf meal as their only source of protein responded favorably when cystine was added to the ration. Similar results were obtained by Kellermann (14) who found that rats fed alfalfa as the only source of protein suffered from

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a deficiency which was immediately relieved by the addition of 0.1 per cent l-cystine. Smuts and Marais (21) found that the addition of 0.2 per cent cystine to an all alfalfa diet fed to rats increased markedly the biological value of the alfalfa protein from 67 to 87. Marais and Smuts (17) reported more recently that methionine was a better supplement than cystine to alfalfa protein but that the best results were obtained when both methionine and cystine were fed.

Wright and Haag (24) reported that the addition of 0.4 per cent l-cystine to the diet of female rats receiving 9 per cent alfalfa protein markedly enhanced the lactation-promoting properties of the ration. In a more recent report, Haag and Wright (9) concluded that cystine and methionine served primarily in making sulphur-deficient rations nutritionally adequate rather than acting as unique lactation stimulants.

In view of the above investigations it appears that the cause of low milk production of our cows on an all alfalfa hay ration might be due to a deficiency of cystine. The object of the present paper is to report the value of cystine as a specific nutrient when fed to lactating cows as a supplement to an all alfalfa hay ration.

#### METHODS

Five cows, two Holsteins, Nos. A7 and A14, two Brown Swiss, Nos. 237 and 239, and one Jersey, No. 77, which had been maintained on an all alfalfa hay ration as their only source of protein and energy since calving, were used in this study. The cows were fed all of the first cutting, No. 2 alfalfa hay that they would eat. The all-roughage ration was supplemented with salt and in some cases with bone meal. Each cow was paired with normally fed cows in the herd to check any marked variation in milk or fat production which might be attributed to environment. Since these data do not show any significant variations, they are not presented. The cows were fed and cared for twice daily and weighed each day. They received their feed while stanchioned in the barn but they were turned out in a dry lot between milking periods. The milk produced by each cow was weighed after each milking and an aliquot sample of the milk (preserved with 5 drops of formalin) was composited over a 3-day period for a butterfat determination. Fat-corrected milk (F.C.M.) was determined by the Gaines formula (5).

The cystine was forcibly fed to each cow in gelatin capsules to insure complete ingestion. The cystine fed to cows Nos. A7 and 239 was prepared from human hair by the method of Gortner and Hoffman (6) while that fed to the other three cows was purchased from a reliable drug manufacturing company.

Corn was fed to four cows and barley to one cow in place of isocaloric quantities of alfalfa or corn starch following the feeding of cystine in order to determine whether or not the cows had the inherent ability to produce more milk when a cereal grain supplemented the alfalfa ration. All of the

cows used in this experiment were past the peak of their production on the all alfalfa hay ration and the rate of production was declining.

The average values obtained from the analysis of the two hays used in this study are shown in table 1. The coefficients of digestibility, digestible

TABLE 1

*Proximate composition of the two alfalfa hays used in this investigation*

	Alfalfa (A) 1st cutting fed to cow No. 239	Alfalfa (B)-light grass 1st cutting fed to cows Nos. A7, A14, 77 and 237
	%	%
Moisture	17.22	11.57
Protein	14.94	10.81
Ether extract	1.16	1.21
Crude fiber	33.50	35.96
N.F.E.	32.43	35.02

protein and total digestible nutrients obtained for cows Nos. A17, A23, A14 and 237 are shown in table 2. These data are based on 10-day collection

TABLE 2

*Coefficients of digestibility, digestible protein and total digestible nutrients of the two alfalfa hays*

Alfalfa No.	Cow No.	Coefficients of digestibility				Digestible protein	Total digestible nutrients
		Protein	Ether extract	Crude fiber	N.F.E.		
		%	%	%	%	%	%
(A)	A 18	71.1	17.0	46.0	62.7	10.6	46.8
	A 23	67.4	8.0	48.9	67.9	7.2	47.0
	Average	69.2	12.5	47.5	65.3	8.9	46.9
(B)	A 14	64.1	45.6	51.4	58.6	6.5	44.4
	237	59.8	28.1	50.2	54.5	6.9	47.2
	Average	62.0	36.8	50.8	56.5	6.7	45.8

periods. Aliquot samples of feces and urine were taken each day, preserved with hydrochloric acid, and saved for chemical analysis. The nitrogen

TABLE 3

*Nitrogen metabolism data of cows receiving alfalfa hay (B)*

Cow No.	Weight	Milk	Intake		Nitrogen outgo				Balance
			Hay	Nitro- gen	Feces	Urine	Milk	Total	
	kg.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
A 14	605.4	12,442	19,688	346.0	126.8	166.6	54.4	347.8	- 1.8
237	524.5	9,426	17,417	297.7	119.7	145.5	50.1	315.3	- 17.6

TABLE 4  
Effect of supplementing an all alfalfa hay or alfalfa-starch ration with cystine and with corn or barley\*

Cow No.	Experi-mental period	In milk	Body weight	Milk	Test	Fat	F.C.M.	Alfalfa con- sumed	Digestible protein		Total digestible nutrients		Remarks
									Rec.	Req.	Rec.	Req.	
237	days	days†	lb.	lb.	%	lb.	lb.	lb.	lb.	lb.	lb.	lb.	Alfalfa alone
	15	102	1159	20.9	4.2	0.87	21.4	39.7	2.66	1.79	18.2	16.0	“ plus 20 gm. cystine per day
	15	117	1151	22.3	3.8	0.86	21.8	38.7	2.59	1.80	17.7	16.0	“ alone
	6	132	1151	20.3	3.9	0.79	20.0	38.7	2.59	1.72	17.7	15.4	“ plus 9 lb. corn per day
A14	15	138	1143	25.0	3.6	0.91	23.6	24.4	2.28	1.89	18.4	16.5	Alfalfa alone
	15	167	1340	27.0	3.3	0.88	24.1	44.8	3.00	2.05	20.5	18.1	“ plus 20 gm. cystine per day
	15	182	1351	28.3	3.1	0.87	24.4	44.5	2.98	2.07	20.4	18.2	“ alone
	6	197	1356	25.9	3.2	0.84	23.0	44.6	2.99	2.00	20.4	17.8	“ plus 9 lb. corn per day
239	15	203	1337	31.8	3.0	0.97	27.2	30.0	2.65	2.21	21.0	19.0	Alfalfa alone
	12	74	1170	23.5	3.9	0.91	23.1	41.4	4.24	1.88	19.2	16.6	“ plus 20 gm. cystine per day
	12	86	1170	23.8	3.9	0.92	23.4	41.9	4.29	1.90	19.4	16.7	“ alone
	9	98	1143	23.7	3.9	0.93	23.4	41.2	4.22	1.88	19.1	16.5	“ plus 9 lb. corn per day
77	18	107	1116	28.2	3.7	1.04	26.8	27.0	3.41	2.07	19.7	17.4	Alfalfa alone
	12	121	764	18.0	5.1	0.93	21.1	26.3	1.76	1.53	12.5	13.1	“ plus 6 lb. glucose per day
	12	133	768	17.7	4.9	0.87	20.1	26.4	1.77	1.49	18.1	12.8	“ plus 6 lb. starch per day
	12	145	767	16.9	4.8	0.82	19.0	27.6	1.85	1.44	18.4	12.5	“ plus starch plus 20 gm. cystine per day
A7	15	157	784	16.5	5.1	0.84	19.1	27.2	1.82	1.45	18.2	12.6	“ plus 6 lb. starch per day
	6	172	792	17.1	5.4	0.92	20.7	28.0	1.88	1.53	18.5	13.2	“ plus 6 lb. corn per day
	12	178	796	18.3	5.2	0.95	21.6	28.0	2.31	1.58	18.2	13.5	Alfalfa alone
	15	95	1165	25.7	2.8	0.73	21.2	39.1	2.62	1.83	18.1	15.9	“ plus 40 gm. cystine per day
A7	12	110	1185	16.0	3.1	0.50	13.9	34.9	2.34	1.45	16.1	13.7	“ alone
	24	122	1157	20.1	2.9	0.59	16.9	38.4	2.57	1.59	17.8	14.5	“ plus 7.5 lb. barley per day
	15	146	1152	24.1	2.7	0.66	19.6	25.0	2.38	1.72	17.5	15.3	

\* The original experimental data were compiled by 3-day periods whereas the above values represent the mean values obtained for each experimental period.

† At beginning of experimental period.

metabolism data for cows Nos. A14 and 237 are presented in table 3. The values obtained for digestible protein and total digestible nutrients were secured from the metabolism trials and these data were used to calculate the intake of digestible protein and total digestible nutrients reported in table 4.

#### RESULTS AND DISCUSSION

The experimental data obtained from this experiment are summarized in table 4. These data include body weight, days in milk, milk and fat production, hay consumption and the total digestible nutrient requirement of each cow and the actual amount each cow received per day during each period. The digestible protein and total digestible nutrients for corn and barley were obtained from Morrison (20) whereas the total digestible nutrients for corn starch and glucose were estimated. As shown in table 4, all of the cows were consuming sufficient total digestible nutrients to meet their requirements for maintenance and milk production, with the exception of cow No. 77 during the initial alfalfa feeding period. Most of the cows were fed more than two pounds of total digestible nutrients in excess of the Morrison (20) feeding standard recommended for good cows under usual conditions.

The data presented in table 4 indicate that the ingestion of 20 grams of cystine per day by cows which had received an all alfalfa hay ration since calving aided in maintaining milk and fat production but did not increase milk production significantly. Cows Nos. 237, A14, and 239 had an average increase in fat-corrected milk of 0.4, 0.3 and 0.3 pounds per day, respectively. The test had a tendency to decrease but the amount of fat produced was not changed significantly. The ingestion of 20 grams of cystine had no effect on the consumption of alfalfa hay. After the cystine had been removed from the ration the fat-corrected milk decreased 1.8, 1.4 and 0.0 pounds per day, respectively. There was a tendency for the test to increase and fat production to decrease. The replacement of 15 pounds of alfalfa with 9 pounds of corn markedly increased the production of butterfat, milk and fat-corrected milk, and decreased the test.

Cow No. 77 was used to study the effect of additional available energy in the ration on milk production. The results show that the addition of glucose, corn starch or corn starch and cystine to an all alfalfa hay ration had no lactation-stimulating effect. A more favorable influence was noted after cystine had been omitted from the alfalfa-starch ration but the addition of six pounds of corn in place of six pounds of corn starch was even more favorable for the production of milk and fat than during the corn starch and cystine feeding period or the initial alfalfa feeding period.

The addition of 20 grams of cystine per day to cows Nos. 237, A14, 239, and 77 did not affect appetite or body weight although Wright and Haag (24) reported that the addition of 0.1 to 0.4 per cent cystine added to the

diet of lactating rats receiving 9 per cent alfalfa protein usually increased the food intake, increased the milk yields, and reduced body weight losses. Daggs and Lidfeldt (3) and Haag and Wright (9) reported that the addition of cystine and methionine to sulfur-deficient diets of lactating rats stimulated lactation. The failure of cystine to increase milk production significantly in cows on an all alfalfa ration may be explained on the basis of a higher percentage of protein in the ration (10.8 to 14.0 per cent) and the somewhat higher coefficient of digestibility of the protein of alfalfa by cows than by rats. Dougherty (4) has suggested that the failure of cystine to increase milk production in dairy cows may be due to its complete or nearly complete destruction in the rumen. This concept is not in complete harmony with the results obtained by feeding cystine (20 grams) as indicated in table 4, because the decline in milk and fat production was checked and a slight improvement in milk yield was obtained. Whether the improvement in milk production can be attributed to the cystine supplement or to a stimulation in metabolism is not known. Our results indicate, however, that cystine is not the first deficiency of an all alfalfa hay ration or of a ration of alfalfa and glucose or corn starch. The possibility of the synthesis of methionine by the rumen flora cannot be ruled out entirely although Woodman and Evans (23) have shown that cystine is not synthesized in the rumen.

The results obtained from cow No. A7, which received 40 grams of cystine per day as a supplement to an all alfalfa ration, are summarized also in table 4. The cow's appetite was not affected during the first three days after receiving the cystine supplement but milk production dropped at once from 18.9 pounds of fat-corrected milk per day during the previous 3-day period to 15.1 pounds per day during the first three days of the cystine feeding period. During each subsequent 3-day cystine feeding period the cow's appetite for alfalfa decreased. This was also accompanied by a decrease in milk yield, an increase in fat percentage and an increase in body weight. After the cystine was discontinued, the cow's appetite and milk production increased and body weight and fat percentage decreased. With the exception of the increase in body weight and the decrease in appetite, no toxic symptoms were manifested. Wright (25) has reported that toxic symptoms were observed in young rats when they received 1 per cent cystine in their diet, but that mature rats were less susceptible than the young rats. Since A7 received about 0.2 per cent cystine in the ration and about 0.5 per cent on total digestible nutrients basis it appears that the bovine has a low tolerance for cystine. The replacement of 15 pounds of alfalfa with 7.5 pounds of barley increased milk and fat production markedly over the cystine feeding period and the subsequent all alfalfa feeding period, further reduced the percentage of fat in the milk and increased the fat production significantly, but did not quite equal the results obtained during the initial alfalfa feeding period.

It should be pointed out that in all cases the milk and fat productions were significantly higher during the corn feeding periods than during the alfalfa feeding periods preceding the ingestion of 20 grams of cystine, which serves to emphasize the fact that the cows had the ability to increase their production against the rapid decline in lactation on the all alfalfa ration provided the necessary lactation-stimulating nutrients were present in the ration.

The increase in milk production obtained when either corn or barley was fed as a supplement to the alfalfa ration confirms the early work of Hart and Humphrey (10) who observed that cows receiving a ration of alfalfa and corn starch declined in milk production but that milk production could be stimulated by changing the cows to a ration of corn, corn gluten feed and corn stover. Marais and Smuts (18) found that the corn grain protein has a fairly high biological value but that the combination of alfalfa and corn proteins supplement each other to a marked degree. Corn is reported to be low in cystine (2) but it contains the necessary factor or factors for efficient milk production when fed as a supplement to an all alfalfa hay ration.

#### SUMMARY AND CONCLUSIONS

1. Five lactating cows which had received alfalfa hay as the sole ration since calving and which had declined in milk production to the point where they were consuming larger amounts of total digestible nutrients than were required by a liberal standard were used in this investigation.

2. The addition of 20 grams of l-cystine per day to the ration of four cows appeared to check the rapid decline in milk and fat production obtained on the all alfalfa hay ration but did not increase milk production significantly.

3. The addition of 40 grams of l-cystine per day to the ration of one cow produced a sharp drop in milk and fat production. The consumption of hay was decreased but body weight increased.

4. The replacement of part of the alfalfa hay with isocaloric amounts of corn produced significant increases in milk and fat production over the initial alfalfa feeding period and the subsequent cystine feeding period.

5. The results of this experiment indicate that cystine is not the first deficiency of an all alfalfa hay ration or of an alfalfa and corn starch ration.

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# A STATISTICAL STUDY OF THE INFLUENCE OF MOISTURE AND ACIDITY ON THE PALATABILITY AND FERMENTATION LOSSES OF ENSILED HAY CROPS<sup>1</sup>

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Since the recent awakened interest in the preservation of hay crops in the silo, we have made about 175 lots of experimental silage at the Beltsville Research Center, with a view to determining the most efficient, practicable, and economical methods of making silage from hay crops.

Most of the silages were made in small wooden silos 4 feet in diameter by 8 feet high and holding about 1 ton each, but some were made in larger silos of paper-lined slatted fence or of concrete. The small silos have proved very useful for this type of work. The quantity in each silo was sufficient for reliable tests of palatability and still not so much as to preclude the practicability of the accurate weighing in and weighing out of all the material. The quantities were also small enough so that all the conditions surrounding the tests could be kept identical except the treatment to be tried. The use of small silos made it possible to try a greater number of methods, and also to make a greater number of replications, than would have been possible with large silos.

Some investigators have claimed that typical silage cannot be made in silos holding only 1 ton. It does appear that the radiation of heat from a small silo prevents the temperature from rising as high and from persisting as long as it would in a larger silo. Furthermore, weighting the top, even at the rate of 40 pounds to the square foot in order to simulate conditions in a deeper silo, still does not cause as much pressure on the silage as would occur in the lower part of a silo of ordinary size. In spite of these differences we have found that the silages preserved in small silos have all the characteristics of normal silage and cannot be distinguished by appearance or odor from those made by similar methods in larger silos. Therefore, we maintain that the most feasible way to try out different methods in well-controlled experiments is by the use of small silos, also that the comparative results of different treatments in small silos will be similar to those that would be obtained in large silos.

## PROCEDURE IN MAKING AND FEEDING THE SILAGE

The chopped crop was always blown into a pile near the small silos. As few as 2 or as many as 12 silos were filled at one time with identical material

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<sup>1</sup> All chemical determinations were made by C. G. Melin, Junior Chemist, Bureau of Dairy Industry.

but with different treatments. The material was weighed in baskets holding 50 pounds each, and one or two basketfuls were placed in each silo in succession. When molasses or acid was used it was added with a sprinkling can after every 100 pounds of chopped material. When crops wilted in the field were compared with unwilted crops the silos were filled at different times, but care was exercised to have wilting the only important variable. When chopped dry hay was used to reduce the percentage of moisture it was mixed with each 100 pounds of the chopped green crop before it was put in the silo. A sample for compositing was taken from the pile every time 100 pounds had been put in each of the silos. The composite sample was analyzed for moisture, nitrogen, and carotene. Since some nitrogen and considerable carotene are lost in drying, the practice was to make the determinations of these constituents on the moist material.

When the silos were nearly full a layer of tarred paper was placed on top of the chopped material and 200 to 400 pounds more chopped material was placed on top of the paper. This made it possible to estimate the losses by fermentation, exclusive of the top spoilage. The filled silo was covered with a wooden follower, and weighted with rock at the rate of 40 pounds per square foot of area.

After 1 to 6 months the silos were opened, one at a time, and the silage fed at once as the sole ration, or with only a few pounds of grain, to 3 or 4 cows for about 6 days in order to see how much they would eat. Individual lots of the silages under comparison were always fed to the same cows, and if any grain was fed, which was seldom, the quantity remained unchanged. Samples of silage for compositing were taken at 5 different depths. Analyses were made for the same constituents and in the same way as when the crop was put in the silo.

#### STATISTICAL ANALYSIS

The first step in making the statistical analysis was to assemble the data into 3 tables. Table 1 shows a comparison of silages with different contents of moisture; table 2 a comparison of silage having a high pH value with silage having a lower pH value in which the lower pH value was brought about by addition of molasses; table 3, a comparison of silage having a high pH with silage having a low pH in which the low pH was brought about by the addition of hydrochloric and sulfuric acids. Since the silages on both sides of each table are the same, except for the treatment at the time the silos were filled, comparisons to determine the influence of the treatment can properly be made only from left to right and not up and down.

The silages were all free of mold and decay. The odor showed that except for certain of the untreated high-moisture legumes, the silages had undergone a desirable type of fermentation. Some of the bad smelling silages lacked palatability, but some of the good smelling acid-treated silages were even more unpalatable.

*Influence of moisture content on the palatability of silage as judged by the quantity of dry matter dairy cows consumed and on the losses of dry matter, protein and carotene in the silo*  
(Comparisons to be made from left to right and not up and down)

Kind of crop put in the silo	Higher moisture				Lower moisture			
	Moisture	Dry matter consumed per cow per day	Losses in the silo exclusive of top spoilage		Moisture	Dry matter consumed per cow per day	Losses in the silo exclusive of top spoilage	
			Dry matter	Protein			Dry matter	Protein
	per cent	pounds	per cent	per cent	per cent	pounds	per cent	per cent
Orchard grass	81.8	19.8	14.0		57.5	23.4	26.0	
Crabgrass, pigeon grass, alfalfa	64.0	17.6	25.0		28.0	21.6	27.0	
Crabgrass, pigeon grass, alfalfa	67.0	19.5	27.0		28.0	21.6	27.0	
Orchard grass rowen	71.3	16.7	0.2	+ 2.5	51.1	18.6	0.6	+ 5.4
Kentucky bluegrass	66.4	21.8	6.1	+ 3.4	20.6	23.0	3.4	+ 0.2
Alfalfa	60.0	16.6	3.6	15.0	40.2	17.6	1.0	+ 1.9
Alfalfa	75.6*	17.7	8.4	+ 0.8	26.0	29.4	2.4	+ 11.5
Alfalfa + HCl and H <sub>2</sub> SO <sub>4</sub>	75.9	10.3	8.3	10.3	36.5	18.5	6.8	+ 0.2
Alfalfa + 3% molasses	75.2*	19.4	10.0	8.4	27.7	31.0	2.3	+ 6.0
Soybeans	70.9	22.2	5.1	+ 3.6	42.4	24.0	1.1	+ 3.7
Alfalfa	71.6	28.9	7.5	1.2	65.1	30.3	6.3	+ 3.2
Alfalfa	71.6	28.9	7.5	1.2	37.0	31.6	0.6	+ 6.4
Alfalfa	74.1*	23.5	9.6	3.6	58.9	26.3	2.6	+ 4.5
<i>Lespedeza sericea</i>	66.6	21.7	8.2	+ 1.3	42.1	22.0	8.3	9.6
<i>Lespedeza sericea</i> + 2.5% molasses	65.8	22.6	10.0	1.3	44.4	22.3	12.5	4.3
<i>Lespedeza sericea</i> + 5.0% molasses	65.9	23.6	7.9	+ 2.9	44.0	22.2	10.4	2.8
Alfalfa	73.5*	13.7	4.2	3.6	66.2†	21.5	9.8	+ 1.5
Oats (slatted fence silo)	73.4	18.3	19.4	14.0	61.6	23.6	10.6	15.9
Oats + 2% molasses (slatted fence silo)	74.2	17.5	12.9	22.1	64.3	21.9	10.7	7.0
Alfalfa (14' × 42' silo)	69.2	24.1	14.0		48.5	27.1	5.3	
Alfalfa (14' × 42' silo)	69.2	24.1	14.0		37.2	25.3	7.7	
Alfalfa + 4% molasses (14' × 42' silo)	67.4	24.9	11.2		41.1	26.3	2.5	
Alfalfa + 4% molasses (14' × 42' silo)	67.4	24.9	11.2		34.4	26.5	13.2	
Alfalfa	72.9‡	28.7	6.7	9.7	57.5	34.0	5.6	1.4
Alfalfa	72.9‡	28.7	6.7	9.7	65.8§	31.0	5.3	3.9
Average	70.6	21.4	10.3	4.8	45.0	24.8	8.3	2.8

\* Offectionable odor. All other silages in this table had a good odor and were free of mold and decay.

† Moisture reduced by adding 19% of dry alfalfa hay.

‡ Odor slightly off.

§ Moisture reduced by adding 15% dry alfalfa hay.

TABLE 2

*Influence of pH values on palatability and on losses of dry matter, protein and carotene in the silo when a reduced pH is brought about by the addition of molasses*  
(Comparisons to be made from left to right and not up and down)

Kind of crop put in the silo	Higher pH				Lower pH								
	pH	Moisture	Dry mat-ter con-sumed per cow per day	Losses in the silo exclusive of top spoilage		pH	Moisture	Dry mat-ter con-sumed per cow per day	Losses in the silo exclusive of top spoilage				
				Dry matter	Carotene				Dry matter	Carotene			
		per cent	pounds	per cent	per cent		per cent	pounds	per cent	per cent			
Kentucky bluegrass	5.52	66.4	21.8	6.1	+ 3.4	14.1	Ky. bluegrass + 3% molasses	5.36	63.1	23.6	5.6	3.4	11.2
Alfalfa	5.30	74.4	14.0	12.1	20.3	6.1	Alfalfa + 3% molasses	4.93	73.9	13.5	12.7	19.9	40.4
Alfalfa*	5.48	75.6	17.7	8.4	+ 0.8	+ 9.2	Alfalfa + 3% molasses*	5.13	75.2	19.4	10.0	8.4	21.1
Alfalfa	5.20	26.0	29.4	2.4	11.5	52.2	Alfalfa + 3% molasses	5.12	27.7	31.0	2.3	6.0	51.1
Soybeans	5.04	70.9	22.2	5.1	+ 3.6	36.9	Soybeans + 3% molasses	4.66	67.3	22.7	9.9	11.0	38.7
Alfalfa	4.48	71.6	28.9	7.5	1.2	67.0	Alfalfa + 4% molasses	4.07	70.6	31.2	9.2	6.4	34.4
Soybeans	4.85	71.4	28.3	5.9	6.0	16.8	Soybeans + 4% molasses	4.09	69.4	29.5	5.2	12.1	3.9
Alfalfa and crab grass	4.36	75.0	22.1	4.9	18.8	62.2	Alfalfa and crab grass + 4% molasses	3.85	73.6	27.0	3.2	12.7	48.3
Lespedeza sericea	4.38	66.6	21.7	8.2	+ 1.3	19.3	Lespedeza sericea + 2.5% molasses	4.10	65.8	22.6	10.0	1.3	23.3
Lespedeza sericea	4.38	66.6	21.7	8.2	+ 1.3	19.3	Lespedeza sericea + 5.0% molasses	4.06	65.9	23.6	7.9	+ 2.9	4.8
Lespedeza sericea	4.85	42.1	22.0	8.2	9.6	51.5	Lespedeza sericea + 2.5% molasses	4.32	44.4	22.3	12.5	4.3	30.7
Lespedeza sericea	4.85	42.1	22.0	8.2	9.6	51.5	Lespedeza sericea + 5.0% molasses	4.30	44.0	22.2	10.4	2.8	25.9
Soybeans*	5.32	78.7	16.6	17.3	15.7	15.1	Soybeans + 4% molasses	4.20	75.8	23.5	13.6	5.5	10.9
Oats (slatted fence silo)	4.07	73.4	18.3	19.4	14.0	33.9	Oats + 2.2% molasses (slatted fence silo)	3.64	74.2	17.5	12.9	22.1	5.9
Oats (slatted fence silo)	4.31	61.6	23.6	10.6	15.9	37.4	Oats + 3.0% molasses (slatted fence silo)	4.16	64.3	21.9	10.7	7.0	33.6
Alfalfa (14' x 42' silo)	4.70	69.2	24.1	14.0		13.0	Alfalfa + 3.4% molasses (14' x 42' silo)	3.94	67.4	24.9	11.2		6.3
Alfalfa (14' x 42' silo)	4.70	48.5	27.1	5.3		25.6	Alfalfa + 4.2% molasses (14' x 42' silo)	4.69	41.1	26.3	2.5		26.2
Alfalfa (14' x 42' silo)	4.71	37.2	25.3	7.7		33.5	Alfalfa + 4.3% molasses (14' x 42' silo)	4.65	34.4	26.5	13.2		25.1
Alfalfa	3.98	72.9	28.7	6.7	9.7	33.9	Alfalfa + 4% molasses	3.65	71.3	30.6	1.4	4.0	4.2
Average	4.76	62.6	22.9	8.7	7.6	30.5	Average	4.36	61.5	24.2	8.6	7.8	23.5

\* Objectionable odor.

TABLE 3

*Influence of pH values on palatability and on losses of dry matter, protein, and carotene in the silo when the pH is brought to 4.2 or lower by the addition of hydrochloric and sulphuric acids*

(Comparisons to be made from left to right and not up and down)

Higher pH					Lower pH								
Kind of crop put in the silo	pH	Mois- ture	Dry matter con- sumed per cow per day	Losses in the silo exclu- sive of top spoilage			pH	Mois- ture	Dry matter con- sumed per cow per day	Losses in the silo exclu- sive of top spoilage			
				Dry matter	Pro- tein	Caro- tene				Dry matter	Pro- tein	Caro- tene	
	per cent	per cent	pounds	per cent	per cent	per cent		per cent	pounds	per cent	per cent	per cent	
Orchard grass rowen	4.45	71.3	16.7	0.2	+ 2.5	19.4	Orchard grass rowen + 6% 2N acid	4.02	72.6	18.3	3.5	+ 12.2	12.6
Kentucky bluegrass	5.52	66.4	21.8	6.1	+ 3.4	14.1	Ky. bluegrass + 10% 2N acid						
Alfalfa	5.30	74.4	14.0	12.1	20.3	6.1	Alfalfa + 10% 2N acid	3.49	65.4	14.2	1.9	+ 5.0	6.2
Alfalfa*	5.48	75.6	17.7	8.4	+ 0.8	+ 9.2	Alfalfa + 9% 2N acid	3.55	74.2	8.6	6.6	+ 2.0	0.9
Alfalfa	5.20	26.0	29.4	2.4	11.5	52.2	Alfalfa + 14% 2N acid	3.66	75.9	18.3	11.4	10.3	+ 14.8
Alfalfa	4.83	59.3	28.1	6.7	2.5	55.4	Alfalfa + 10% 2N HCl	3.34	36.5	18.5	8.6	+ 0.2	41.3
Alfalfa*	5.43	74.1	23.5	9.6	3.6	42.9	Alfalfa + 6% 2N acid	3.93	61.7	20.3	5.4	6.7	34.8
Alfalfa*	5.43	74.1	23.5	9.6	3.6	42.9	Alfalfa + 8% 2N acid	4.06	73.8	18.6	1.8	+ 1.4	12.0
Soybeans	5.12	71.0	28.3	9.8	12.5	21.6	Soybeans + 4% 2N acid	3.72	73.8	13.3	+ 1.3	+ 6.4	11.5
Soybeans	5.12	71.0	28.3	9.8	12.5	21.6	Soybeans + 6% 2N acid	4.20	70.8	25.1	5.6	13.2	5.0
Soybeans	5.12	71.0	28.3	9.8	12.5	21.6	Soybeans + 8% 2N acid	3.19	71.0	16.2	7.3	17.8	6.6
Soybeans	5.12	71.0	28.3	9.8	12.5	21.6	Average	3.95	71.1	15.4	4.0	11.3	+ 14.1
Average	5.18	66.8	23.6	7.7	6.6	26.2		3.66	67.9	16.3	5.0	2.9	9.3

The F test for significance<sup>2</sup> was applied to 3 comparative treatments: (1) high moisture vs. low moisture, (2) high pH vs. lower pH brought about by the addition of molasses, and (3) high pH vs. low pH brought about by the addition of hydrochloric and sulfuric acids. (See table 4.)

TABLE 4  
*F tests for significance*

	Dry matter consumed	Losses in the silo		
		Dry matter	Protein	Carotene
High moisture vs. low moisture				
F values found	25.74	4.06	1.05	10.36
F values required at odds of 99: 1	7.82	7.82	8.40	8.18
F values required at odds of 19: 1	4.26	4.26	4.45	4.38
Significance in favor of	Low moisture	Neither	Neither	High moisture
Higher pH vs. lower pH through addition of molasses				
F values found	7.89	58.33*	200*	2.98
F values required at odds of 99: 1	8.28			8.28
F values required at odds of 19: 1	4.41			4.41
Significance in favor of	Lower pH	Neither	Neither	Neither
High pH vs. low pH through addition of hydrochloric and sulphuric acids				
F values found	32.70	2.98	1.67	25.11
F values required at odds of 99: 1	10.04	10.04	10.04	10.04
F values required at odds of 19: 1	4.96	4.96	4.96	4.96
Significance in favor of	High pH	Neither	Neither	Low pH

\*Mean square of error exceeded mean square of treatment.

The data presented in table 1 show that a reduction of the moisture content of high-moisture crops improves the quality of the silage, as judged by the odor and dry matter consumed, without at the same time increasing the losses of dry matter or protein. But they show also that reducing the moisture content by wilting or by adding dry hay increases the loss of carotene in the silo, and there is, of course, a greater loss of carotene in the field when the crop is allowed to wilt. Furthermore, we found that low-moisture silages do not keep as well when exposed to the air as high-moisture silages, and they must be fed out more rapidly after the silo is opened to prevent spoilage.

On the other hand, high-moisture silages exert a greater lateral pressure on the silo walls than low-moisture silages; and they are likely to leak juice, which is not only a nuisance but is also destructive to any sort of concrete or metal with which it comes in contact. Moreover, high-moisture crops require more labor.

<sup>2</sup> Snedecor, Geo. W. Statistical Methods, pp. 171-218. Collegiate Press, Inc., Ames, Iowa. 1937.

We feel that one should wilt the crop only enough to prevent leakage. If wilted only to this extent the odor will be good, the carotene will be well preserved, and there will be no need for any so-called preservative.

Silages made from low-moisture material have about the same pH values as silages made from high-moisture material, indicating that reducing the moisture content of the crop has no material effect on the acidity of the silage. The fact that low-moisture legumes make good silages shows that the development of considerable acidity is not necessary. Our work, and that of others, shows that legume silages that have a high-moisture content and a low acidity are likely to be ill-smelling, but if the acidity is high the odor of the silage will be good. In other words, a legume crop with a high-moisture content should be ensiled with such treatment as will produce considerable acidity in order to make a good-smelling silage. A wilted crop, however, can have a low acidity and still make good silage.

Our experience indicates that 68 per cent of moisture in legumes is about the dividing line so far as both leakage and odor are concerned. Legumes with more moisture should either be wilted or mixed with a dry material, or the acidity should be increased by the addition of acid or some material from which acid is formed.

The addition of molasses improves the odor of high-moisture legume silages and the palatability of all silages. It has no significant effect upon the losses that occur in the silo. No one appears to know just what products are formed from the molasses in the silo, nor what the nutritive values of these products are. It is impossible to say, therefore, whether or not a farmer gets back in feeding value 100 cents for every dollar he spends for molasses. In spite of this, the use of molasses is a good practice with high-moisture legumes, because of the improvement it brings about in the odor and palatability of the silage.

The addition of hydrochloric and sulfuric acids is not to be advised no matter how perfectly they appear to preserve the nutrients of a crop. No silo made of metal, tile, or concrete can long withstand the action of these acids. The silage is definitely unpalatable. The efficient preservation of carotene may be more apparent than real, as the Nutrition Division of the Bureau of Dairy Industry has found that much of the material passing for carotene in acidified silage is not true carotene. Investigations with silage to which acids have been added show the necessity for conducting actual feeding tests before making definite recommendations regarding the practical application of a method on dairy farms.





# THE USE OF ULTRAVIOLET RAYS IN THE CHEESE FACTORY AND STORAGE ROOM<sup>1</sup>

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The relative inexpensiveness of low-pressure ultraviolet lamps has awakened interest in their possible commercial value. Their maximum ultraviolet emission is in the 2500–2600Å region, which according to Gates (5) is the region of maximum lethal activity. Broadbent (1) gave installation data on the lamps as well as initial and upkeep cost. Several investigators have studied the lamps' lethal action on air-borne bacteria. On this subject the work of Sharp (9) and Whisler (11) deserves particular attention.

Because of the enthusiastic claims of some investigators, such as Garrett and Arnold (4), attention was given to the possible use of these low-pressure lamps in the dairy industry. Since mold contamination of cheese is a problem, tests were made on the value of the lamps in controlling the mold. Price (8), using the carbon lamp, had been unable to prevent the molding of cheese; but this new source might possibly prove more effective than the carbon lamp.

## EXPERIMENTAL

### *Air-Borne Mold Spores*

Six 15-watt hot cathode lamps were placed in a cheese-curing room measuring 15'7" × 12'2" × 11'3". Air circulation was effected by two fans with 6-inch blades. Data on the condition of the room were collected by placing open Petri plates containing hardened Sabouraud's agar at four places in the room. These plates were exposed for 15 minutes and incubated at 30° C. for 72 hours. Observations covering a year failed to show any effective action—any control of the undesirable surface mold formation.

In studies to determine the efficacy of the lamp in killing air-borne mold spores, a 15-watt lamp was installed in a 3-inch tube. With the tube in a vertical position, mold spores were dislodged from a Petri plate and allowed to fall by the lamp. These spores, having been collected on sterile filter paper coated with sterile glycerine, were dispersed into distilled water adjusted to a surface tension of 35 dynes with sodium ricinoleate.<sup>2</sup> A total

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<sup>1</sup> The author is indebted to the Committee on the Relation of Electricity to Agriculture, whose grant made this work possible; and to the General Electric Company for the lights used in these studies.

<sup>2</sup> Previous study had shown that this concentration of ricinoleate had no demonstrable effect on the spores, but did aid in dispersing them into the liquid.

count was made with a blood-counting chamber, and a viable count was taken by the plate method, using Sabouraud's agar. Judging from a series of tests with the light burning and the light off, the average percentage kill traceable to the lamp was 10 per cent.

Conceivably, the mold spores in this experiment would be clumped and would thus not allow the best conditions for ultraviolet activity. In the second experiment, therefore, air from contaminated room was pulled through a 3-inch tube containing a 15-watt hot cathode tube. The rate of flow as calculated by a venturi nozzle was 10 feet per minute. The air was circulated for 1 hour around an open Petri plate containing Sabouraud's agar. Tests were made with the lamp turned on and off. The average count with the lamp off was 12 molds per plate; with the lamp operating, 14 molds. With this apparatus it was impossible to secure a slower air flow.

These observations were confined to mold spores (*Penicillium roqueforti*) that have been shown to require 40–80 times as much radiation as *Escherichia coli* for complete killing (7). The data reported in the literature have been largely confined to *Escherichia coli* and do not indicate the value of the lamps on mold studies.

#### THE IRRADIATION OF CHEESE

The paraffined types of cheese were studied. Immediately before being coated, they were exposed to ultraviolet light for 10 minutes. The distance from the cheese surface to the lamp was 10 inches. Three lamps were arranged so that all surfaces of the cheese would be exposed to the rays. A second series of cheeses were similarly exposed for 10 minutes, paraffined, and again irradiated for 10 minutes. Judging from a study of 40 cheeses, exposure to ultraviolet light for the periods used in this study was not effective in preventing mold growth on the surface of the cheese or of the paraffin.

Curran's work (3) indicated that bacterial spores were more sensitive to heat after exposure to ultraviolet light. The experiments described above failed to indicate that the irradiated spores were more susceptible to the hot paraffin than those not irradiated. Possibly by the time of dipping (2–3 days) mycelia may have already penetrated the surface of the cheese. If this were true they would be more resistant to the ultraviolet. According to Tanner (10) the mycelia that had penetrated the surface of agar were very hard to kill.

Conceivably, some of the ultraviolet might penetrate the coating of the cheese and exert a lethal action on the organisms under the paraffin. Although no absolute figure could be secured for the thickness of the paraffin coating, values from 0.4–1.0 mm. were secured. Penetration studies were then made on certain of the materials used in coating or covering the cheese. The following values were obtained:

Coating material tested	Thickness in mm.	Microwatts/cm <sup>2</sup>
None . . . . .	0	2100
Paraffin . . . . .	0.64	370
Beeswax . . . . .	0.56	110
*Pliofilm . . . . .	0.07	800
*Pliofilm . . . . .	0.07	400

\* The pliofilm giving a value of 800  $\mu$  w/cm<sup>2</sup> had no inking, whereas that with the 400  $\mu$  w/cm<sup>2</sup> was selected to contain the maximum inking.

The values in this table were secured by means of a General Electric light meter with a germicidal attachment. This apparatus was described by Luckiesch (6), and the conversion factor from foot candles to micro watts has been given by Buttolph (2).

These data indicated that irradiation before or after paraffining with 15-watt low-pressure mercury-vapor lamps would not be effective in preventing the mold growth on cheese. Coating materials commonly used exerted a marked screening action on the 2537Å ultraviolet light.

According to experiments on canned Cheddar cheese, the lamp failed to prevent mold growth in the canned product even though cans and wrappers were autoclaved.

#### CONCLUSIONS

Ultraviolet light emitted from the 15-watt low-pressure mercury-vapor lamps has not proved effective in decreasing air-borne mold spores in the cheese curing room. Direct irradiation has failed to prevent mold growth on the surface of the cheese.

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# FACTORS AFFECTING THE PASSAGE OF LIQUIDS INTO THE RUMEN OF THE DAIRY CALF. II. ELEVATION OF THE HEAD AS MILK IS CONSUMED\*

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## INTRODUCTION

The accumulation of milk in the rumen and the reticulum of young calves is an abnormal state that is generally considered undesirable physiologically and nutritionally. In order to prevent this anomaly many systems of feeding and management have been devised and advocated. Since the esophageal groove is the only opening into this rumenoreticular cavity, the control of the course taken by the milk into and through the complex stomach of the calf is associated directly with the physiology of this groove. Thus many theories, involving factors from mechanical manipulation to psychic stimulation, have been advanced to explain the functional response of the esophageal groove.

Among the factors that have been assigned rôles in determining the reaction of this groove is the position of the calf's head (up or down) while the milk is being consumed. According to the postulation of Schmoker (3) when a calf suckles, the uplifted head and extended neck draw and close the esophageal groove, thus preventing the entrance of the milk into the two fore compartments of the stomach. Contrariwise, if the calf drinks from an open pail on the ground or floor, its lowered head and relaxed neck fail to close the esophageal groove permitting milk to enter the rumenoreticular cavity. This theory from the viewpoint of either practical calf feeding or fundamental physiology seemed to merit investigation to ascertain its validity.

## EXPERIMENTAL

The problem was studied from the standpoint of anatomy as well as that of physiology. The experimental subjects were dairy calves within the normal milk feeding age, six months or less.

## ANATOMICAL OBSERVATIONS

**Methods.** Young male calves were sacrificed and dissected to study the physical relationships of the esophagus and of the reticular, or esophageal, groove to other organs. Immediately after "knocking" the calf, the esoph-

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ageal groove was exposed by rumenotomy. As the groove was being observed, the head of the calf was moved back and forth several times to positions similar to those maintained while either drinking from a pail (head lowered and neck curved downward) or nursing a cow (head elevated, mouth extended and neck outstretched). Subsequently dissection was continued exposing the esophagus and parts of the thoracic and abdominal organs as shown in figure 1.



FIG. 1. Dissection view of calf showing the comparative length of the esophagus and its position in relation to other organs. T, trachea; E, esophagus; H, heart; L, lung; D, diaphragm; S, spleen; R, rumen; O, abomasum. Insert indicates the relative location of the esophageal groove in the stomach. C, cardia; R-O, reticulo-omasal orifice; Ru, interior rumen wall; Re, interior reticulum wall.

**Results.** The esophageal groove (insert of figure 1) extends from the cardia (C), the distal terminal opening of the esophagus, to the reticulo-omasal orifice (R-O), the opening into the omasal canal. The groove, as normally found in young calves, was closed and twisted in a semi-spiral fashion, but as illustrated in figure 1, it is stretched open to show its relationship to surrounding organs. Though the groove is connected directly with the esophagus (E), these two organs are considered to be morphologically independent. From a purely anatomical viewpoint, it is difficult to relate the positions of the head and neck of the calf to the functioning of the esophageal groove.

This point of view was substantiated by the failure of the manipulations, previously described, to alter the position of the groove lips, the proximity of which determines whether or not the groove is closed. Thus from ana-

tomical observations it would seem that the reaction of the esophageal groove is regulated by factors other than the mechanical movements and the positions of the head and neck of the calf.

#### PHYSIOLOGICAL OBSERVATIONS

**Methods.** The experimental animals were rumen-fistula calves, including ten Holsteins, four Jerseys and two Guernseys. From a managerial standpoint the only deviation from practiced methods was the continuous confinement of these calves within a barn. The rations, qualitatively and quantitatively, were considered typical for dairy calves.

As a means of determining the interrelationship of the elevation of the head (up or down) and the system of feeding (nipple or open pail) to the course followed by the milk in its passage through the esophageal groove, the feeding positions and systems illustrated in figure 2 were used.



FIG. 2. Relative position of calf feeders used in studying the effect of head and neck elevation on the course followed by consumed milk in its passage through the esophageal groove. (A) elevated nipple, (B) elevated open, (C) lowered nipple and (D) lowered open.

In the lowered position the calf feeders were placed upon the level surface on which the calf stood while consuming the milk; whereas in the elevated position the height of the pail was adjusted so that when the calf was feeding, its poll was at approximately the same level as the point of its withers. Positions either higher or lower than these were considered abnormal and impracticable.

Two types of feeder pails were used, the nipple and the open. In the nipple system the regular Coyner feeder\* was employed in the elevated

\* Sold by Armour and Company, U. S. Stock Yards, Chicago, Ill.



position (A), but in the lowered position (C), the Coyner nipple was inserted into a hole in the center of a circular board cut to fit inside an open pail. The board, floating on the surface of the milk, maintained the nipple in an upright position. Thus the calf was able to suck the milk while holding its head and neck in a position similar to that assumed when drinking from a lowered open pail (D).

The normal nursing position (A) could not be simulated entirely in drinking from an open elevated pail (B). In this case the height could be adjusted properly, but obviously the calf could not drink conveniently with its mouth extended upward at a slight angle as is done in nursing. Therefore, in order to facilitate consumption from the open elevated feeder (B) a part of the side was cut from the pail.

The reactions of the esophageal groove and the path followed by the milk were determined either by direct visual inspection (9) or by palpation of the groove. These observations were made regularly immediately before feeding and during the time that the calf was consuming the milk.

Since the anatomical studies indicated that the mechanical movements of the head and neck had no effect on the reaction of the esophageal groove, the same manipulations and observations were repeated on the live fistulated calves, first in a normal state and later under general anesthesia.

In order to obtain information on the origin and the transmission of the stimuli that activate the esophageal groove, the left vagus in each of several anesthetized calves was exposed near the pharynx and was repeatedly irritated with a "tetanizing" electric current. This stimulation was applied with the head and neck in positions of lowered-pail feeding and elevated-pail feeding, respectively.

*Results.* The recorded observations indicating the relation of the elevation of the head and of the system of feeding, respectively, to the frequency with which consumed milk entered the rumenoreticular cavity are summarized in tables 1, 2, 3 and 4. The significance of the various differences was determined by the application of the chi-square test. The chi-square values as shown in tables 1 and 2 reveal a very significant difference

TABLE 1

*Summary of observations on esophageal groove passage of milk when it was consumed from two different types of feeding pails at floor level*

System of feeding	Course of consumed milk		Total	Frequency of entrance into rumen
	Passed rumen	Entered rumen		
Open pail	<i>observations</i> 525	<i>observations</i> 361	<i>observations</i> 886	<i>per cent</i> 40.7
Nipple pail	307	17	324	5.2

Difference = 35.5%,  $\chi^2 = 13.91$ ,  $P = 0.001$

TABLE 2

*Summary of observations on esophageal groove passage of milk when it was consumed from two different types of feeding pails in an elevated position*

System of feeding	Course of consumed milk		Total	Frequency of entrance into rumen
	Passed rumen	Entered rumen		
Open pail	<i>observations</i> 369	<i>observations</i> 305	<i>observations</i> 674	<i>per cent</i> 45.3
Nipple pail	835	43	878	4.9

Difference = 40.4%,  $\chi^2 = 356.96$ ,  $P = < 0.001$

between nipple and open pail feeding when the milk was consumed from either an elevated or a lowered position. Invariably, drinking milk from an open pail resulted in greater spillage (frequency and quantity) into the rumenoreticular cavity than when consumed via nipple. On the contrary, the data presented in tables 3 and 4 indicate that the relative elevation of

TABLE 3

*Summary of observations on esophageal groove passage of milk when it was consumed through a nipple at different positions of elevation*

Position of feeding pail	Course of consumed milk		Total	Frequency of entrance into rumen
	Passed rumen	Entered rumen		
Floor level	<i>observations</i> 307	<i>observations</i> 17	<i>observations</i> 324	<i>per cent</i> 5.2
Elevated	835	43	878	4.9

Difference = 0.3%,  $\chi^2 = 0.06$ ,  $P = 0.80$

TABLE 4

*Summary of observations on esophageal groove passage of milk when it was consumed from an open pail at different positions of elevation*

Position of feeding pail	Course of consumed milk		Total	Frequency of entrance into rumen
	Passed rumen	Entered rumen		
Floor level	<i>observations</i> 525	<i>observations</i> 361	<i>observations</i> 886	<i>per cent</i> 40.7
Elevated	369	305	674	45.3

Difference = 4.6%,  $\chi^2 = 3.18$ ,  $P = 0.10$

the head and neck, irrespective of system of feeding, did not affect significantly the frequency of passage of milk into the rumen.

Further supporting evidence resulted from observations of the reactions of the esophageal groove. The movements of the head and neck, within the

limits previously indicated, did not affect the opening and closing of the groove. This was the case not only with anaesthetized animals but also with unaesthetized ones. Raising and lowering the head while the calf was either sucking or drinking likewise failed to alter the state of the groove.

The stimulus applied to the vagus nerve resulted in the closure of the open relaxed esophageal groove and in marked motility of the rumen. Relaxing and subsequent opening of the groove followed a few seconds after the cessation of the stimulation. The position of the head and neck again had no effect on the reaction of the groove.

#### DISCUSSION

The relation of the elevation of the calf's head, within practical limits, to the frequency of milk spillage into the two fore compartments of the stomach and the resulting effect on the health of the calf have been somewhat exaggerated. This fact is evidenced by the results in the foregoing anatomical and physiological investigations considered in the light of previous observations (9).

Even a cursory study of the morphology and the anatomy of the esophagus and esophageal groove would preclude erroneous conclusions relative to the effect of head elevation on the course followed by the milk. The esophagus is comparatively thin-walled, flexible and very dilatable. According to Sisson and Grossman (4) the muscle tissue is striped, consisting of two strata of spiral fiber, except near the stomach, where the tissues are longitudinal and circular. The esophagus has no terminal dilation and no part in the abdominal cavity. The reticular, or esophageal, groove being a part of the stomach obviously is anatomically distinct from the esophagus. The nature of the esophagus considered in conjunction with its relationship to the spiral-form groove renders it difficult to attribute the opening and closing of this organ to changes in the elevation of the head.

The physiological observations substantiated the postulations based on the anatomical studies. Only extremes in the elevations of the open feeder pail, resulting in abnormal deglutition, affected the course of the milk. In the few observations made the volume of the swallows seemed to be one of the primary factors involved. In all instances the groove closed irrespective of the posture assumed. When the milk was drunk from an abnormal and impractical height, the swallows were small and no milk escaped into the rumen, but when milk was drunk from a subnormal level, below foot-base, the volume of the individual swallows seemed to be sufficiently large to force the liquid between the lips of the groove into the rumenoreticular cavity. Elevation changes within the limits considered practical did not alter the volume of the swallow to any marked extent.

Consumption of milk through a rubber nipple resulted, with rare exception, in direct passage of the milk into the distal stomach compartment,

the abomasum. This is in accord with previous findings (8, 9). Evidently the nursing stimulus, the size of the swallows and other closely related regulatory factors take precedence over the position assumed by the calf while ingesting the milk.

However, Watson (5) concluded that in the case of lambs the act of sucking did not affect the course of the milk in the stomach. His deduction is based on the observation that when sheep had become accustomed to quenching their thirst by sucking water from a rubber nipple, the replacement of water by either milk- or water-barium sulphate suspension resulted, on a majority of occasions, in the passage of the suspensions into the fore compartments of the stomach and not into the abomasum.

The observed reactions of the esophageal groove in fistulated calves are apparently at variance with Watson's results. In the case of calves beyond a month of age, tap water consumed via rubber nipple frequently entered the rumenoreticular cavity (9) but always in considerably less quantity than when drunk directly from an open pail. Furthermore, feeding milk through the same nipple-feeder immediately following water consumption did not result in passage of the milk into the rumen and reticulum; even though ingestion of much water, according to Wester (7), tends to lessen the reflex irritability.

In considering the physiological responses of the groove, cognizance must be taken of the fact that there are many variations in different calves and in the same individual at various times. As indicated in data previously presented (9) and further confirmed in this investigation, there is a wide range in the frequency and volume of spillage into the rumenoreticular cavity when calves ingested the milk from open pails. Evidently these variations may be attributed to an interaction of diverse factors, all of which probably have not been recognized. Thus it becomes difficult to appraise the effect of any one factor *per se*; consequently seemingly discordant results frequently appear.

Evidence has been adduced (2, 6, 7) indicating that the functioning of the groove is a reflex response elicited by either a mechanical or a chemical stimulant coming in contact with certain regions of the mouth and pharynx. From these receptors the stimulus, according to Wester (7), is transmitted through the vagi to the esophageal groove. The electrical irritation of the left vagus in foregoing experimental subjects, in accord with Dougherty's (1) observations on a mature cow, motilized the rumen. This response was accompanied by contraction of the lips of the esophageal groove. Normally, however, the closure of the groove in young calves is not associated with marked rumen motility. The nature of the irritant probably was one factor affecting the extent of the reaction. The results indicate that the vagi are paths over which certain stimuli may reach the esophageal groove. Whether or not there are other means of transmission remains a moot question.

The positions of the head and neck do not affect the stimulus transmission and the resulting reaction.

#### SUMMARY

1. Anatomical studies suggested that the elevation and the mechanical manipulation of the head and neck of calves, within limits that may be employed in practical feeding, are not primary factors affecting the functioning of the esophageal groove.

2. This suggestion was substantiated from observations of rumen-fistula calves fed milk either through a rubber nipple at different levels, floor and elevated, or from open pails at these levels.

3. Though the elevation of the head and neck was not an important factor affecting the extent of milk passage into the rumenoreticular cavity, the type of feeder, nipple or open, was very significant.

4. Irrespective of feeder level, milk rarely entered the rumen when consumed via nipple but frequently entered when drunk from an open pail.

5. The vagi are paths over which certain stimuli may be transmitted to the esophageal groove, but the transmission and resulting esophageal groove reaction are unaffected by positions of the head and neck.

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# STUDIES ON THE CHEMICAL COMPOSITION OF THE BLOOD OF DAIRY CATTLE. III. THE NORMAL CONCENTRATION OF INORGANIC PHOSPHORUS IN THE WHOLE BLOOD OF DAIRY CATTLE AND FACTORS AFFECTING IT<sup>1</sup>

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The level of inorganic phosphorus in the blood of dairy cattle is especially sensitive to low levels of phosphorus intake (11, 16, 21, 22) to deficiencies of Vitamin D in the ration, or to exposure of animals to sunshine or ultra violet light (2, 10, 18). If it may be assumed that a ration just capable of supporting a normal (maximal) concentration of inorganic phosphorus in the blood is adequate in phosphorus for all prevailing functions, providing of course, the ration is well balanced in all other respects, the blood inorganic phosphorus level may be readily applied in the determination of the minimum phosphorus requirements of cattle. In order to determine the minimum phosphorus requirements of cattle by this means it is necessary to know the normal level of blood composition of animals at various ages (15, 21) and the effect of such factors as pregnancy, lactation and various environmental conditions.

Normal values for the inorganic phosphorus content of blood serum or plasma have been reported by Palmer, Cunningham and Eckles (15), Anderson, Galey and Pratt (1), Haag and Jones (7) and Eveleth, Eveleth and Walsh (4). Similar values for whole blood are relatively few. The inorganic phosphorus content of the blood is subject to wide fluctuations from day to day and from period to period (8, 15, 17). Values obtained on composite samples of whole blood show considerably less variation from time to time than similar values obtained on single samples of plasma or whole blood (8). Stare and Elvehjem (19) studied the phosphorus partition of the blood of rachitic and non-rachitic calves and came to the conclusion that whole blood was preferred for this determination. Whole blood has been used by Godden and Allcroft (6) in a study of the changes in the composition of cow's blood at the time of calving and a comparison of the blood of the calf with that of the dam. Theiler, Green and Du Toit (20), Malan, Green and Du Toit (13), and more recently Otto (14) used whole blood in studies of phosphorus metabolism of cattle in South Africa. In previous reports data showing the effects of age, phosphorus intake, gestation and lactation on the concentration of inorganic phosphorus in the whole blood of dairy heifers have been pre-

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sented (21, 22). More recently similar data for mature milking cows have been presented by Johnson (12).

The purpose of this paper is to report normal values for the concentration of inorganic phosphorus in the whole blood of dairy cattle during the periods of growth, gestation and lactation at different seasons of the year and with and without exposure to sunlight.

#### EXPERIMENTAL

Several important considerations governed the routine of this investigation. An effort was made to obtain comparable representative samples of whole blood from animals which were handled in a normal manner as well as animals maintained under strictly experimental conditions.

*Methods of sampling and analysis.* For this study composite samples of whole blood consisting of 10 ml. aliquots from six daily samples taken on alternate days over a period of ten days as previously described (8) were analyzed for inorganic phosphorus. All samples were handled in a uniform manner and analyzed for inorganic phosphorus by the Fiske and Subbarow (5) method. Potassium oxalate was used as an anti-coagulant. Determinations were made at one or two month intervals during the period the animals were under observation. Each value, therefore, represents an average of six daily samples for each individual animal at that particular time.

*Description of animals and rations.* Data for the period of growth up to the time of first calving were obtained on 26 Holstein heifer calves. Many of these animals were under observation for the entire period; however, some were not started on the experiment until several months of age while others were removed from the experiment from time to time for various reasons not related to this study.

The calves received whole milk for the first four to six weeks at which time they were gradually changed to skim milk. Concentrates and hay were offered as soon as the calves would consume them. The skim milk was gradually discontinued when the heifers were approximately six months of age. Eight of the calves were fed a normal mixed ration composed of yellow corn meal, ground oats, wheat bran, corn gluten meal and salt with timothy hay as roughage. This ration supplied ample quantities of calcium and phosphorus to meet the requirements of growing dairy heifers. These heifers were confined continuously to a well lighted and well ventilated experimental barn. The other eighteen heifer calves were fed an experimental ration composed of alfalfa hay, alfalfa leaf meal, corn starch and chipped corn sugar supplemented with salt and steamed bone meal. The heifers in this group were permitted to run outside in dry lot except during inclement weather.

During lactation data were obtained on three groups of milking Holstein

cows. Group 1 consisted of 12 cows in the regular milking herd. This group received the regular herd concentrate ration which is supplemented with salt and 2 per cent steamed bone meal. Alfalfa hay and corn silage were fed as roughages. The cows in this group were on pasture in season. The cows were selected in the spring of 1936 on the basis of producing at least 40 pounds of milk per day. Several of the animals produced from 50 to 60 pounds and two from 65 to 70 pounds per day at the peak of production during the lactation period. Inorganic phosphorus was determined at bi-monthly intervals for a period of two lactations or approximately two years. Nine of the cows were under observation throughout the entire period, whereas three of them were removed from the herd during the period. Group 2 consisted of 16 cows in the experimental herd. Five of the animals in this group received a ration composed of alfalfa hay and yellow corn meal supplemented with salt and steamed bone meal. The other 11 animals received an experimental alfalfa ration composed of alfalfa hay and a concentrate mixture made up of equal parts by weight of chipped corn sugar and alfalfa leaf meal supplemented with 1.5 per cent salt and 2 per cent steamed bone meal. All animals in Group 2 were confined to the experimental barn continuously during the lactation period. Group 3, consisting of 5 animals in the experimental herd, received a ration of timothy hay and a concentrate mixture composed of yellow corn meal, soybean oil meal, salt, and 2 per cent steamed bone meal. The animals in this group were permitted to run outside in dry lot except during inclement weather.

During the growing period hay was fed at the rate of 1.5 pounds per 100 pounds body weight per day. The remainder of the digestible crude protein and total digestible nutrients required to meet the average of the Morrison (9) feeding standard for growing dairy heifers was supplied by the concentrate mixture. During the lactation period all animals were fed hay at the rate of about 2 pounds per 100 pounds body weight per day and the concentrate mixture according to the rate of production. Digestible crude protein and total digestible nutrients supplied were calculated by the Morrison feeding standard for milking cows.

## RESULTS

*Effect of rations.* There was no indication of any difference in the inorganic phosphorus content of the blood as a result of the ration fed in these experiments. The rations were composed of good alfalfa or timothy hay and a concentrate mixture supplying liberal quantities of phosphorus.

*Effect of age.* The data obtained during the period of growth up to about 28 months of age are presented statistically in table 1 and summarized graphically in figure 1. The heifers were bred when they were about eighteen months of age. Several of them failed to conceive promptly thus permitting an opportunity to obtain values for non-pregnant heifers for



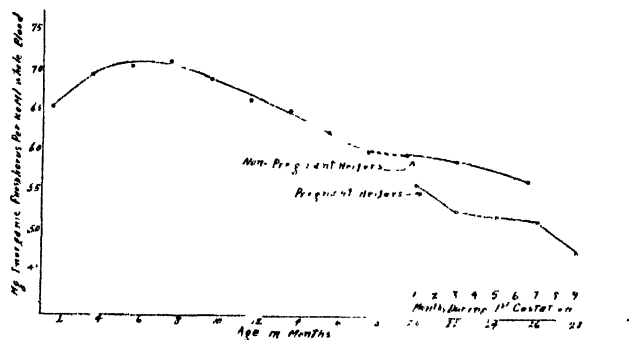


FIG. 1. Effect of age and gestation on the inorganic phosphorus content in the whole blood of growing dairy heifers.

comparison with those of about the same age as heifers which were pregnant. In table 1 is recorded the number of animals under observation for each time interval, the number of daily samples taken, the number of composite samples analyzed, the mean and standard error, the standard deviation, and the coefficient of variation. It may be observed that the inorganic phosphorus content of the blood increased until the heifers were about six to eight months of age and then gradually decreased as they grew older.

*Effect of gestation.* The data presented in table 2 and in figure 1 show the effect of both an increase in age and pregnancy of growing heifers during the period of first gestation. It may be seen in table 2 that there was a considerable decrease in the inorganic blood phosphorus from the beginning of gestation with an average of 5.65 mg. to the ninth month just preceding

TABLE 1  
*The normal concentration of inorganic phosphorus in the whole blood of growing dairy heifers*

Age groups	Animals observed	Daily samples taken	Composite samples analyzed	Mean and standard error	Standard deviation	Coefficient of variation
<i>months</i>	<i>no.</i>	<i>no.</i>	<i>no.</i>	<i>mg. per 100 ml. whole blood</i>		<i>%</i>
1.0- 2.5	6	126	21	6.58 ± 0.10	0.46	6.99
2.5- 4.5	13	126	21	6.97 ± 0.11	0.52	7.46
4.5- 6.5	19	162	27	7.08 ± 0.11	0.53	7.49
6.5- 8.5	19	150	25	7.15 ± 0.09	0.44	6.15
8.5-10.5	19	156	26	6.94 ± 0.11	0.56	8.10
10.5-12.5	20	168	28	6.68 ± 0.08	0.43	6.44
12.5-14.5	20	168	28	6.56 ± 0.09	0.49	7.47
14.5-16.5	20	156	26	6.29 ± 0.11	0.55	8.74
16.5-18.5	18	144	24	6.06 ± 0.14	0.69	11.39
18.5-20.5*	18	144	24	6.03 ± 0.13	0.65	10.77
20.5-23.5*	11	138	23	5.95 ± 0.08	0.40	6.72
23.5-27.5*	6	114	19	5.68 ± 0.10	0.44	7.75
Average					0.51	7.96

\* Non-pregnant heifers.

TABLE 2

*Effect of stage of first gestation on the inorganic phosphorus of whole blood of dairy heifers*

Stage of first gestation	Animals observed	Daily samples taken	Composite samples analyzed	Mean and standard error	Standard deviation	Coefficient of variation
<i>months</i>	<i>no.</i>	<i>no.</i>	<i>no.</i>	<i>mg. per 100 ml. whole blood</i>		<i>%</i>
0-2	16	108	18	5.65 ± 0.11	0.48	8.50
2-4	16	102	17	5.32 ± 0.11	0.45	8.46
4-6	16	102	17	5.30 ± 0.17	0.70	13.20
6-8	16	120	20	5.19 ± 0.14	0.61	11.75
9th*	11	66	11	4.81 ± 0.17	0.56	11.64
Average					0.56	10.71

\* Average age at time of first calving 28 months.

calving with an average of 4.81 mg. per 100 ml. of whole blood. Figure 1 also shows that there was a gradual decline in blood composition of non-pregnant heifers from 18 to 27 months of age, but the decline was much more pronounced in the case of pregnant heifers of approximately the same age. This fact was particularly noticeable in the decided drop during the last month of the gestation period.

*Effect of the number and stage of lactation.* Data for all animals during the milking period according to the number and stage of lactation are

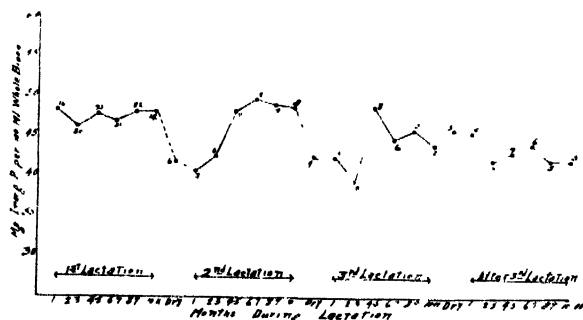


FIG. 2. Effect of the number and stage of lactation on the inorganic phosphorus content of whole blood. Numerals indicate the number of composite samples analyzed and averaged.

presented in table 3 and in figure 2. The data have also been summarized statistically according to lactations and recorded in table 4. It may be observed in figure 2 that the inorganic phosphorus of the blood showed practically no change throughout the first lactation period. The number of animals studied beyond the first lactation was somewhat smaller, but there appeared to be a definite tendency for the inorganic phosphorus to show a drop near the end of the second gestation period. The inorganic phosphorus continued at a low level during the first two or three months of the second lactation and then increased to about the same level as during the

TABLE 3

*Effect of the number and stage of lactation on the concentration of inorganic phosphorus in whole blood of dairy cows*

Stage of lactation	No. of animals observed	No. of daily samples taken	No. of composite samples analyzed	Mg. inorganic phosphorus per 100 ml. of whole blood		
				Minimum	Maximum	Mean
First Lactation						
During 1st month	16	96	16	3.80	5.91	4.86
2nd and 3rd "	23	180	30	3.75	5.94	4.65
4th and 5th "	24	210	35	3.32	6.51	4.81
6th and 7th "	23	186	31	3.57	6.06	4.73
8th and 9th "	22	132	22	3.65	5.70	4.84
10th and 11th "	14	84	14	4.17	5.76	4.86
Dry period	6	36	6	3.90	4.47	4.21
Second Lactation						
During 1st month	3	18	3	3.84	4.50	4.09
2nd and 3rd "	6	36	6	4.01	4.54	4.29
4th and 5th "	9	66	11	4.10	5.84	4.86
6th and 7th "	11	78	13	4.17	6.30	5.00
8th and 9th "	10	60	10	4.47	6.14	4.94
10th and 11th "	8	48	8	4.28	5.96	4.91
Dry period	4	24	4	3.39	5.01	4.26
Third Lactation						
During 1st month	5	30	5	3.30	5.03	4.27
2nd and 3rd "	7	42	7	3.30	4.58	3.98
4th and 5th "	5	48	8	3.77	5.39	4.92
6th and 7th "	5	36	6	3.90	5.70	4.51
8th and 9th "	5	30	5	3.86	5.45	4.63
Dry period	5	30	5	3.71	5.62	4.64
Four or More Lactations						
During 1st month	4	24	4	4.00	5.22	4.56
2nd and 3rd "	8	48	8	3.62	4.67	4.23
4th and 5th "	6	42	7	3.71	4.97	4.34
6th and 7th "	5	30	5	3.56	5.45	4.45
8th and 9th "	4	24	4	3.62	4.82	4.25
10th and 11th "	3	18	3	4.05	4.47	4.25

TABLE 4

*Effect of number of lactations on the inorganic phosphorus content of whole blood of dairy cows*

Lactations	Animals observed	Daily samples taken	Composite samples analyzed	Mean and standard error	Standard deviation	Coefficient of variation
no.	no.	no.	no.	mg. per 100 ml. whole blood		%
1	25	888	148	4.81 ± 0.04	0.44	9.15
2	13	306	51	4.80 ± 0.08	0.55	11.46
3	8	198	33	4.35 ± 0.10	0.57	13.10
4 or more	7	186	31	4.34 ± 0.09	0.52	11.98
Combined		1578	263	4.69 ± 0.04	0.61	13.01

TABLE 5  
*Effect of season on the inorganic phosphorus of whole blood of lactating animals*  
(Mg. per 100 ml. of blood)

Animal No.	Calving Dates	Group 1. Normal Milking Herd—Pasture in Season												
		June '36	August	October	December	February '37	April	June	July	September	November	January '38	February	April
176	Mar. 36, Feb. 37, Jan. 38	5.24	4.89	5.03	4.34	4.31 C	3.62	4.23	4.97	3.45	4.82	4.47	4.53	4.52
305	Dec. 35, Mar. 37, Feb. 38	4.04	4.23	4.05	3.44	3.92 D	3.98	3.95	3.56	3.62	4.22	4.29 D	4.82	4.67
347	Feb. 36, Feb. 37, Jan. 38	5.84	5.16	4.77	4.82	4.50 C	4.34	5.18	5.39	5.70	5.45	5.62 D	5.22	4.67
355	Feb. 36, Feb. 37, Jan. 38	5.18	4.98	4.76	4.50	3.97 C	3.30	4.46	4.46	5.15	5.36	5.21 D	4.38	4.68
357	Feb. 36	5.64	5.01	4.52	4.10	4.10 D								
358	Dec. 35, Mar. 37, Apr. 38	4.98	4.73	4.64	4.10	4.08 D	3.84	4.11	4.13	4.07	3.86	4.22	4.71 D	
360	Dec. 35, Dec. 36, Jan. 38	5.03	4.59	4.28	3.39 D	3.69	3.77	3.90	4.25	4.47	4.16	3.96 D	3.66	3.71
361	Jan. 35, Dec. 36, Feb. 38	5.69	5.33	5.07 D	4.56 D	4.35	4.70	4.58	4.70	5.36	5.37	5.01 D	5.03	4.58
362	Dec. 35, Oct. 36, Nov. 37	4.98	4.74	4.02 C	4.04	4.17	3.98	4.01	3.71 D	4.00	3.77	3.95	4.17	
365	Feb. 36	5.39												
373	Mar. 36, Mar. 37, Jan. 38	4.98	5.16	4.50	3.65	4.20 D	4.01	4.56	4.46	4.89	4.47	4.55 D	3.83	4.00
400	Aug. 36			4.34	3.96	4.43	4.29	4.49	4.19					
Average		5.14	4.93	4.54	4.12	4.16	3.98	4.35	4.38	4.75	4.61	4.59	4.48	4.36

Group 2. Experimental Herd—Kept in Barn											
January '36	March	May	July	September	October	December	February '37	April	June	July	September
389	4.65	4.35	4.29	4.71	3.90 D	3.84	4.07	4.10	4.17	5.30	5.96
390	3.89	4.61	5.22	5.76	4.23 D	4.50	4.44	4.46	4.67	6.30	6.14
391	4.19	4.91	4.76	4.71	4.67	3.93 D	3.93	4.54	4.23	5.82	
393	4.38	4.65	5.40	4.94	5.48	5.15	4.47	4.35	4.49	5.61	
Average	4.28	4.63	4.92	5.03	4.57	4.36	4.23	4.36	4.39	5.76	6.05

first lactation. During the latter part of the third gestation period the inorganic phosphorus showed another decrease similar to that observed during the latter part of the previous gestation period. The inorganic phosphorus was again low during the first two or three months of the third lactation, but increased to about the former level during the latter part of the lactation period. After the third lactation no significant change in blood composition was observed.

*Effect of season during growth and lactation.* The data obtained for growing heifers from the age of one month to the time of first calving did not indicate that there were any differences in the composition of the blood depending upon the season of the year in which the calves were born. Neither did it seem to make any difference whether the calves were confined to the barn continuously or allowed to run outside in favorable weather.

In table 5 are presented data for the 12 animals in group 1 in the regular milking herd and for 4 animals of group 2 in the experimental herd. The cows in Group 1, with the exception of numbers 361 and 400, were beyond their first lactation when this study was begun, whereas the 4 cows in Group 2 were started at the beginning of the first lactation and continued through the second lactation. The other 12 cows in Group 2, and 5 cows in Group 3, were observed during the first lactation only. The cows in Group 1 were out on pasture from the first half of May until about the fifteenth of October each year. The 4 animals in Group 2 were confined to the barn continuously.

The first composite sample of blood for the animals in Group 1 was taken in 1936. It may be observed that there was a decrease in the average amount of inorganic phosphorus in the blood from 5.14 mg. in June 1936 to 3.98 mg. in April 1937, after which there was an increase reaching a peak of 4.75 mg. in September followed by another decline until April 1938 when the study was discontinued. In other words, there was a tendency for the blood inorganic phosphorus to be lower during the winter and spring than during the summer and early fall. This was not due entirely to the fact that the cows were outside and on pasture. This tendency may be observed also in the case of the 4 animals in Group 2, table 5 which were kept in the barn continuously. The blood inorganic phosphorus was relatively low in March 1936 with an average of 4.28 mg. per 100 ml. after which there was a gradual rise to 5.03 mg. in September. Following the peak of 5.03 mg. in September 1936 there was a gradual decline to 4.23 mg. in February 1937, then a gradual rise to 5.76 mg. in July. Two of the cows averaged 6.05 mg. in September when the experiment was discontinued.

#### DISCUSSION

Data obtained in this investigation indicate that for heifer calves receiving liberal quantities of phosphorus and such amounts of vitamin D as

occurred normally in the ration, age is the most important factor affecting the inorganic phosphorus of the blood. There is first an increase in the inorganic phosphorus up to about the sixth or seventh month and then a marked decrease as the animals increase in age up to the time of first calving. There is also a slight trend downward with an increase in the number of lactations until about the third or fourth lactation. These results are very similar to results obtained by Johnson (12).

The individual effect of such factors as the stage of lactation, milk production, gestation and season of the year on the inorganic phosphorus of the blood is difficult to evaluate. The cows were usually bred so as to calve during the winter months when on dry feed and for the most part confined to the barn. As has been pointed out, pregnancy caused a definite lowering of the inorganic phosphorus during the latter part of the gestation period in heifers bred for the first time. There is also a tendency for cows to show low blood inorganic phosphorus during the latter part of the second and third gestation when they are usually dry. Eckles and associates (3) found that there is a marked increase in the phosphorus requirements of a cow during the last 6 weeks to 2 months previous to parturition and that provisions must be made for this to prevent disaster to the mother following calving. Blood inorganic phosphorus was usually lower for the first two or three months of the second and third lactation periods than during the latter part of these lactations. This tendency for the blood phosphorus to be lower for a month or so before calving and at the beginning of the lactation period may represent a shift temporarily in the equilibrium between the phosphorus available and that required at that particular time, and not a deficiency of phosphorus in the ration. These animals were all receiving liberal quantities of phosphorus in the ration since the ration was supplemented with bone meal.

There was a tendency for milking cows to show a lower level of blood inorganic phosphorus during the winter and early spring than during the summer and fall, which may be explained in part on the basis of the effect of pregnancy and early lactation since most of the cows were bred to calve during the winter and early spring months. Fifty-one composite samples taken from 12 cows in the regular milking herd from November 1 to April 30 averaged 4.29 mg. whereas 56 samples taken from the same animals from May 1 to October 30 averaged 4.70 mg. per 100 ml. of whole blood. An average of 4.29 mg. per 100 ml. of whole blood from November until April compares very closely with results reported by Johnson (12). He obtained average values for the inorganic phosphorus in whole blood of milking and dry cows of 4.28 and 4.35 mg. per 100 ml., respectively. In this work all samples were taken during the spring when the animals had been away from pasture for 4 months or longer.

There is some indication that the concentration of inorganic phosphorus

in the blood of lactating cows under normal conditions is, in part, characteristic of the individual animal. Animals with average blood values either above or below the herd value for one lactation were likely to maintain the same relative position for the next lactation.

#### SUMMARY AND CONCLUSIONS

Inorganic phosphorus was determined in more than 600 composite samples of whole blood taken from 59 cows and heifers in normal states of health and nutrition.

When growing dairy heifers were fed rations composed of good alfalfa or timothy hay and a concentrate mixture supplying liberal quantities of phosphorus and such amounts of vitamin D as occurred normally in the ration, age was the most important factor affecting the concentration of inorganic phosphorus in the blood.

The inorganic phosphorus in the blood of growing dairy heifers showed an increase with age up to about the seventh or eighth month after which there was a gradual decline as the animals grew older. There was also a decrease in the blood inorganic phosphorus of lactating cows with an increase in the number of lactations up to about the third or fourth lactation.

During the period of first gestation a lower concentration of inorganic phosphorus is present in the blood of heifers than in that of unbred heifers of approximately the same age.

There was no significant change in blood inorganic phosphorus during the first lactation. There was a strong tendency for cows to show low values for inorganic phosphorus during the last 6 or 8 weeks of the second gestation period, and for the first two or three months of the second lactation. Blood phosphorus was also low during the last few weeks of the third gestation period and for a month or so at the beginning of the third lactation. After the third lactation no significant change in blood composition was observed.

Heifer calves receiving concentrates and about 1.5 pounds of alfalfa or timothy hay per 100 pounds body weight, daily, showed no indication that the season of the year born, or the fact that they were confined to the barn continuously or permitted to run outside in favorable weather, had any effect upon the inorganic phosphorus content of the blood.

There was a strong tendency for dairy cows after the first lactation to show a lower concentration of inorganic phosphorus in the blood during the winter and early spring than during the summer and early fall.

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## BLIND HALVES IN A GOAT'S UDDER<sup>1</sup>

C. W. TURNER AND E. R. BEROUSEK

*Department of Dairy Husbandry, University of Missouri, Columbia*

The inability to remove milk from one or more quarters of a cow's udder is experienced occasionally. The term "blind or imperforate" teat or quarter is usually attached to this condition. A study of the literature indicates that there may be several causes of this condition. In some cases the condition arises from a hereditary or developmental defect in the mammary gland, whereas in other cases the defect arises as a result of a local injury, inflammatory or mastitic condition of the teat or gland. It is not always easy to distinguish between these causes.

1. *Imperforate teat.* This is a condition described by Alexander (1) and Billings (2) in which a membrane has formed at the end of the teat. While an anatomical study of this condition was not presented, it would necessarily involve the streak canal. Surgical treatment of this condition is rather simple.

2. *Blocked teat cistern.* A condition is frequently described in which a movable object blocks the inner entrance to the streak canal and thus prevents the removal of milk. If the object cannot be removed or caused to disintegrate by massage, this condition becomes rather exasperating. The following substances have been reported in the teat cistern: (a) coagulated mucus or casein (3); (b) milk calculus or stone (3, 1); (c) warty growths (1).

3. *Membrane separating teat and gland cistern.* The presence of a septum or membrane between the teat and gland cistern has been reported (1, 7). Normal milk secretion would be initiated following calving but the presence of the membrane would prevent the removal of milk from the gland. Foust (4) has presented a series of drawings indicating how this condition could arise in the fetal development of the udder. This condition has also been observed in sheep. Gardner (7) observed three such cases in a series of 26 udders examined.

4. *Closure of primary milk ducts.* Swett *et al.* (6) has presented figures of sections of a number of cow's udders where the obstruction to the removal of milk appeared to be due to an overgrowth of connective tissue or scar tissue around the cistern of the gland and the primary milk ducts leading from the cistern. It was suggested that this condition arose from the early infection of the quarter resulting in the discharge of pus. In calves shortly after birth, the gland consists of a cistern with limited growth of the duct

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<sup>1</sup> Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series, No. 817.

system. An infection at this time with resulting scar tissue production might cause the permanent closure of the ducts but later hormonal stimulation of the ends of the duct system enables the upper secretory part of the gland to grow normally. The gland would thus develop normally during pregnancy but would be found to be blind after calving.

In the Missouri Station herd, a sterile cow which had had chronic mastitis in several quarters was observed some time later, upon the experimental stimulation of milk secretion, to have *three* blind quarters which upon section appeared similar to several of the sections presented by Swett *et al.* As these quarters had milked satisfactorily previously it would appear that in this case the closure of the primary milk ducts had occurred during the period when the cow was dry due to the condition of chronic mastitis (5). On the basis of these observations, it would appear possible for mastitis to cause the closure of the finer ducts in any part of the udder by overgrowth of the surrounding connective tissue.

The object of the present paper is to present a study of the case of a purebred dairy goat from both halves of whose udder milk could not be removed. It seems to differ significantly from previously reported cases in that the absence of milk secretion appears to be due to an embryological developmental closure of the primary milk ducts rather than to an overgrowth of connective tissue resulting from infection.

#### CASE HISTORY

Mrs. C. E. Pope of Springfield, Illinois, reported to the writers that a certain purebred Saanen goat (La Qualité Mi-Favorite, No. 55472) failed to give more than a few cubic centimeters of milk from either half following her first parturition. Thinking that there might be involved an interesting endocrine imbalance, it was suggested that the goat be rebred and if, after the second parturition, a similar condition prevailed, the animal be brought to Columbia for further study.

The animal kidded for the second time on February 4, 1941; however, she was not brought to Columbia until February 22. At that time, gross examination showed each half of the udder to be about the size of a man's fist. Palpation indicated a rather meaty, fibrous condition of the udder. As 18 days had elapsed since parturition without milk removal, one would expect considerable resorption of the milk present at that time.

In order to determine whether the possible endocrine imbalance was related to the growth of the udder, or to the stimulation of milk secretion, the right half of the udder was surgically removed<sup>2</sup> for gross and histological examination. The gland weighed 349 gms. An attempt was then made to inject Bouin's fluid through the teat into the gland. Not more than 10 cc.

<sup>2</sup> The authors are indebted to Dr. A. A. Lewis and E. P. Reineke for aid in removing the half.



FIG. 1. An X-ray photograph of the udder half to which a suspension of barium sulphate had been injected. The cistern of the teat appears normal. The cistern of the gland is restricted to a duct. The primary ducts which normally branch from the cistern of the gland are restricted and end blindly. Secretion from the gland tissue above cannot be removed.



FIG. 2. An X-ray photograph of a normal udder half to which a suspension of barium sulphate had been injected. All parts of the gland communicate with the cistern and duct system.

could be injected into the teat and gland cistern. Beyond the gland cistern there seemed to be definite obstruction to the normal flow of fluid.

The teat therefore was cut lengthwise to note the condition at the point of the obstruction. It was found that the structure of the teat was approximately normal, but at the base of the teat there was a constriction but not a closure at the point where the teat cistern merges into the gland cistern. Above the teat, instead of an enlarged gland cistern, there was a duct from which several branches extended for a short distance into the gland, then ended blindly.

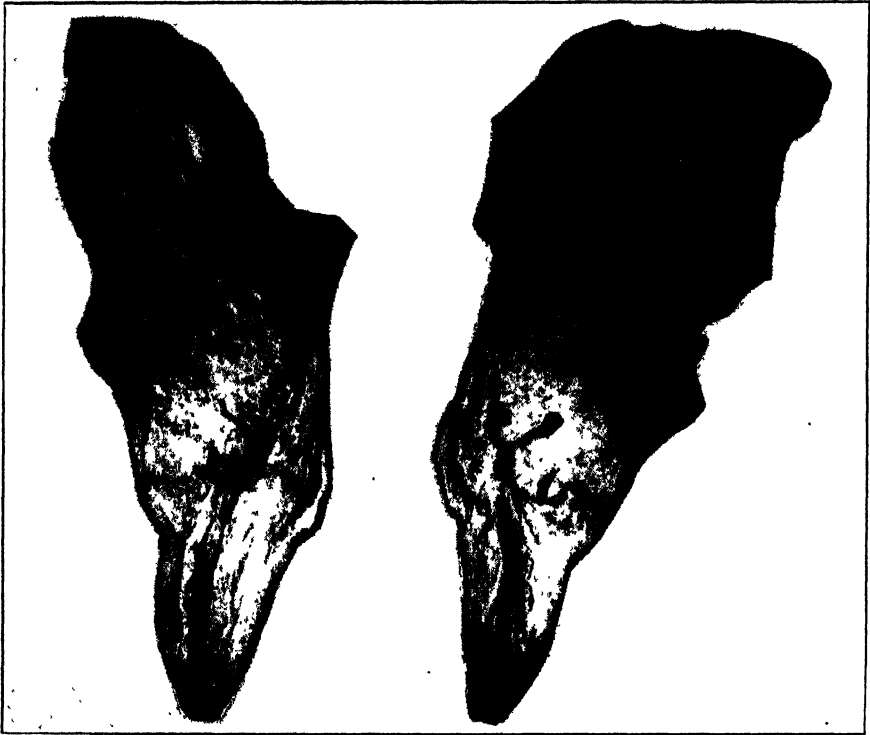


FIG. 3. Dissection of the same udder half as shown in fig. 1 following the injection of a formalin solution to harden the tissue. A perpendicular section shows the normal streak canal, cistern of the teat, duct-like gland cistern, and blind ends of the primary ducts. (For normal goat udder see reference 7.)

The observations up to this point led us to believe that the lack of milk production was due to constrictions of the primary milk ducts preventing the drainage of milk from the gland. That this was true was determined by the examination of fresh thin sections cut from the upper parts of the gland examined under the dissecting microscope and by histological sections.

While the resorption of milk was in a rather advanced stage and the size of the lumina of the alveoli were greatly reduced, there were abundant

signs of milk secretion from normal epithelial cells. No signs of mastitis, such as the excessive presence of leucocytes, were observed in any part of the gland. The strands of connective tissue appeared somewhat larger than normal but this should be expected with resorption of the bulk of the milk.

Sections cut at the point of constriction of one of the primary ducts showed a concave mass of connective tissue underlying the duct epithelium. Beyond this constriction no further evidence of the duct was observed but clumps of alveoli surrounded by rather heavy strands of connective tissue were present.

The animal was kept in our herd and rebred. In November 1941, the animal was sacrificed and the left half removed. The weight of the half was 227 gm. Fluid was again injected into the teat, but as before only about 10 cc. could be held. It was decided to fill the teat cistern with a suspension of barium sulphate and take an X-ray photograph (figs. 1 and 2). The photographs showed a normal cistern of the teat but a duct rather than a gland cistern. Only a few primary ducts were present which extended for a short distance into the gland and then ended blindly. The barium sulphate suspension was removed from the half and a 10 per cent formalin solution was injected into the cistern of the teat as far as it would go. After hardening, the teat and gland were sectioned (fig. 3). The two halves were thus observed to be essentially similar anatomically.

#### DISCUSSION

The present case is believed to differ from the previous described types of "blind" mammary glands in that the constriction and blockage of milk removal are located in the primary ducts rather than between the cistern of the teat and gland cistern. It is true that in dairy cattle a condition similar to this has been described in quarters, which either at an early age of the animal or following several lactations, were infected with mastitis. In such cases there appeared to be an overgrowth of the connective tissue surrounding the primary milk ducts closing the ducts.

The following facts are considered in opposition to the theory that the cause of this condition was due to an early infection of the udder. First, the two halves showed essentially the same condition. Second, the owner of the goat was of the opinion that other goats had not sucked her to set up an infection or irritation. Third, there was no histological evidence of a heavy leucocytic infiltration of the tissue. Fourth, there did not appear to be an overgrowth of the connective tissue around the ducts but rather a blind ending of the ducts.

On the other hand, the evidence in favor of the suggestion that the condition is either inherited or is due to a defective embryological condition follows: First, the owner of the goat studied is acquainted with another goat of similar breeding which also failed to give milk. Whether the anatomical con-

dition is the same is not known. Second, the two halves appeared to have the same defect.

#### SUMMARY AND CONCLUSIONS

A case of a goat is reported which failed to give milk after parturition. It was found that both halves were blind. The gland tissue was capable of secreting milk but the primary milk ducts ended blindly. It is believed that this condition is due to an inherited or developmental defect in the glands rather than to the development of connective tissue overgrowth resulting from mastitic infection.

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# ABSTRACTS OF LITERATURE

## ADVANCE ABSTRACTS OF REPORTS TO APPEAR IN THE JOURNAL OF DAIRY SCIENCE

1. **The Danger of Hydrochloric Acid Gas Poisoning When Testing Salt-Treated Cream.** H. C. HANSEN AND R. S. SNYDER, University of Idaho, Moscow, Idaho.

Samples of salt-treated cream were analyzed according to a modified method developed at the University of Idaho Agricultural Experiment Station. Salted creams of 5 to 13 per cent concentrations, when tested by the Babcock method, released high concentrations of hydrochloric acid gas dangerous to health. Single samples of 7.5, 10 and 13 per cent concentrations released hydrochloric acid gas in amounts above the maximum allowable for prolonged exposure. Sets of 12 to 24 samples in any of the concentrations tested, released hydrochloric acid gas above the maximum allowable for even a short exposure ( $\frac{1}{2}$  to 1 hour). The slow rate of diffusion caused high concentration of gas near the operator thus increasing the danger.

2. **Devices for Measuring Physical Properties of Cheese.** L. A. ROGERS AND G. P. SANDERS, Division of Dairy Research Laboratories, Bureau of Dairy Industry, U. S. Department of Agriculture.

Instruments are described for measuring the firmness of curd at cutting, the elasticity of Swiss cheese, and the plasticity or toughness of Swiss cheese.

The instrument for measuring firmness of curd is a modification of the Hill curd meter but may be used on a vat of milk and automatically records the firmness of the curd in terms of the time required for the cutter to move a definite distance through the curd.

Elasticity is measured by subjecting a disk of cheese of specified dimensions to air pressure. The curvature of the disk under a given air pressure for a given time is indicated by a thickness gauge graduated to read to 1/1000 of an inch.

Plasticity or toughness is evaluated by determining the force required to extrude the cheese through a small orifice in a cylinder. Force is applied to the cheese through a piston supporting a reservoir for water. Water flows into the reservoir until the first particle of cheese forced through the orifice breaks an electric circuit and closes the water valve. The reservoir is graduated to read the weight of water in pounds.

3. **Effect of Holding Cream in the Buying Station upon the Mold Content and Certain Other Quality Factors.** R. W. MORRISON, F. E.

NELSON, AND W. H. MARTIN, Kansas Agricultural Experiment Station.

The applicability of the methylene blue-borax visual mold test for grading cream held for one or two days in the cream station was studied. A preliminary survey of 75 samples showed that the visual mold score increased an average of only 0.03 units. The average increase in titratable acidity was 0.15 per cent of the 46 samples on which acidity was determined.

A more complete study on 38 cans of cream was made during the month of May, 1941. Cream temperature, titratable acidity, visual mold score, mold plate count, yeast plate count and organoleptic grade were determined before and after holding for one or two days in the station. The temperature of the cream usually was between 70° and 80° F., both at the time it was placed in holding and at the time it was shipped. Holding temperatures were maintained somewhat below maximum atmospheric temperatures by the limited use of ice. A tendency for the mold test or the plate count to decrease on samples originally high in mold content and to increase on samples originally low in mold content was observed. Changes in organoleptic grade and acidity were not paralleled by changes of similar magnitude in visual mold score, mold plate count or yeast plate count. Variation in space above the cream, within the limits of usual commercial practice, apparently did not affect the changes observed. The defects which appeared during the holding were of a variety of types, and no relationship between type of defect and other changes was observed.

The results indicate that the visual mold test used as an index of the quality of cream for buttermaking does not reflect the changes which occur during holding in the cream station.

## BOOK REVIEW

4. **Refrigeration, 2nd Edition.** JAMES A. MOYER, State Director of University Extension in Massachusetts, and RAYMOND U. FITZ, Assistant Professor of Mechanical Engineering, Tufts College. Published by McGraw-Hill Book Co., Inc., 330 W. 42nd Street, New York. 538 pages, 291 illustrations. \$5.00.

A general treatment of refrigeration presented in understandable fashion and revised to cover recent refrigeration developments and applications.

Contents:

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| 13. Cold Storage of Foods.                           |   |

L.M.D.

## BACTERIOLOGY

5. A Synthetic Medium for the Cultivation of *Streptococcus Fecalis*.

ROSLYN L. SCHUMAN AND MICHAEL A. FARRELL, Penn. State College, State College, Pa. Jour. Infect. Dis., 69, No. 1: 81-86. 1941.

A completely synthetic medium consisting of pantothenic acid, vitamin B<sub>6</sub>, riboflavin, glucose, a salt mixture, arginine, glutamic acid, methionine, tryptophane, tyrosine, and valine; supported active growth of a strain of *Streptococcus fecalis*, as measured by means of a photoelectric nephelometer. Beta alanine could not replace pantothenic acid in the medium. The addition of other amino acids and accessory growth factors did not measurably increase growth.

J. F.C.

6. The Action of Sulfanilamide upon Hemolytic *Streptococci* Lancefield, Groups A and D, in Growth-promoting and Nongrowth-promoting Media.

ERWIN NETER, Univ. Buffalo, School of Medicine, Buffalo, N. Y. Jour. Infect. Dis., 68, No. 3: 278-284. 1941.

Group A and group D streptococci suspended free of nutrients in a buffered solution and in a similar solution containing 1 per cent of sulfanilamide survived approximately the same length of time in both suspension media, indicating that there was no bacterial effect exerted by sulfanilamide under these conditions. When the suspension media contained growth-promoting nutrients, the medium containing 1 per cent sulfanilamide exhibited marked bacteriostatic action within 2 to 4 hours. With group A streptococci a concentration as low as 0.1 per cent of sulfanilamide in the growth-promoting medium decreased the rate of growth.

J.F.C.

7. Variation in Peroxide Production by Beta Hemolytic *Streptococci*.

FAITH P. HADLEY, PHILIP HADLEY, AND WILLIAM W. LEATHEN, Inst. of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa. Jour. Infect. Dis., 68, No. 3: 264-277. 1941.

Eight type 3 and 2 type 5 strains of group A beta hemolytic streptococci that failed to give evidence of peroxide production by the usual tests, gave rise to peroxide-producing variants when aged for 4 to 10 days on benzidine

blood agar. The variants were stable. They differed from the parent strains in cell morphology, virulence, phagocytability *in vivo*, and in the amount of type- and group-specific substance. There was no difference noted in colony morphology, fermentative ability, and sensitivity to the inhibiting action of hydrogen peroxide. Neither the parent nor the variant strains produced catalase. J.F.C.

**8. Influence of Sulfanilamide on Mucoid and Smooth-phase Cultures of Hemolytic Streptococci in vitro.** PHILIP HADLEY AND FAITH P. HADLEY, Inst. of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa. Jour. Infect. Dis., 68, No. 3: 246-263. 1941.

In broth media a type 5 beta hemolytic streptococcus of Group A in mucoid phase was markedly inhibited at 37° C. in concentrations of sulfanilamide of 1:40,000 or greater. When the incubation temperature was raised to 40° C. the 1:10,000, 1:20,000, and 1:40,000 concentrations were often germicidal for the mucoid phase in 24, 48, and 72 hours respectively. Serial passage at 37° C. and at 40° C. in neopeptone broth containing increasing concentrations of sulfanilamide resulted in progressive transformation from mucoid to smooth phase. The small percentage of remaining mucoid forms continued to possess virulence, but the derived smooth forms were lacking in virulence and did not regain it either by cultivation procedures or by mouse passage. The possible importance of such modification *in vivo* during sulfanilamide therapy is discussed. J.F.C.

**9. Dissociative Aspects of the Bacteriostatic Action of the Sulfonamide Compounds.** RUTH A. MCKINNEY AND RALPH R. MELLON, Inst. of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa. Jour. Infect. Dis., 68, No. 3: 233-245. 1941.

Mice with experimental pneumococcal peritonitis were treated with sulfonamide compounds in less than maximally efficient doses. From such mice a series of intermediate variant pneumococci or "modulations" was isolated. This series of "modulations" ranged from a slightly modified mucoid colony to a minute colony composed of unencapsulated, avirulent organisms. It represented a gradient of diminishing metabolic activity and increasing phagocytability. J.F.C.

**10. An Additional Growth Factor Needed by Some Hemolytic Streptococci.** A. BASS, SAM BERKMAN, AND FELIX SAUNDERS, Univ. Chicago. Jour. Infect. Dis., 68, No. 3: 220-225. 1941.

Using two strains of Lancefield's Group A streptococci as test organisms, the authors tested for necessary accessory growth requirements with a basal medium consisting of hydrolyzed gelatin supplemented with amino acids,

inorganic salts, 0.2 per cent dextrose, and the accessory growth factors nicotinic acid, B-alanine, hemin, i-inositol, cocarboxylase, and riboflavin. The further addition of glutamine, ascorbic acid, pantothenic acid, and B<sub>6</sub> was not sufficient for growth of the test organisms. The addition of an especially prepared extract of yeast resulted in good growth, with less activity resulting from the addition of extracts of spleen, liver, fresh tomato juice, green pepper, banana, and potato. The necessary factor in these extracts was soluble in water and glacial acetic acid, but not in anhydrous solvents or fat solvents. It withstood autoclaving in neutral solutions, but was rapidly destroyed by heat in dilute alkali or acid. It could be precipitated almost completely from concentrated extracts with silver salts. Charcoal was the only effective adsorbent found. Elution from charcoal was difficult and incomplete. Attempts at purification for identification have been unsuccessful. J.F.C.

**11. Isolation of Haemolytic Streptococci from Wounds.** A. E. FRANCIS, London. *Lancet*, 241, No. 6154: 159-160. 1941.

In a series of 300 wound specimens the gentian violet agar (1:500,000) of Garrod showed 50 per cent more positive for hemolytic streptococci than plain blood agar. In a series of 60 specimens containing proteus or *Pseudomonas pyocyanea* but giving negative results for hemolytic streptococci on plain blood agar, 18 yielded hemolytic streptococci when the inoculated plates were covered with a second layer of agar and treated with alcohol according to a modified method of Fry. Also, the phenol agar (1:1,000) of Braun and Schäffer yielded positive results in 11 of 120 specimens when plain blood agar was rendered useless by the presence of proteus or *Pseudomonas pyocyanea*. The use of sodium azide for the suppression of the gram negative organisms was not satisfactory. J.F.C.

**12. The Distribution of Hemolytic Streptococci, Groups A, B, and C, in Human Infections.** LOWELL A. RANTZ AND CHESTER S. KEEFER, Thorndike Memorial Lab., Second and Fourth Med. Services (Harvard), Boston City Hospital, and the Dept. of Med., Harvard Med. School, Boston. *Jour. Infect. Dis.*, 68, No. 2: 128-132. 1941.

Eleven hundred and fifty-nine strains of hemolytic streptococci isolated from human sources were grouped serologically. Of these strains, 1,104 (95.2 per cent) belonged to group A; 19 (1.6 per cent) were group B; 14 (1.2 per cent) were group C; and 22 strains were not classified except to determine that they did not belong to groups A, B, and C. Of the 19 group B strains, 4 appeared to be the primary etiological agents involved in human infections. Some of the remaining 15 group B strains were found in conditions which suggested their etiological relationship, whereas the strains iso-



lated from throats and sputums were of doubtful significance. None of the group C strains appeared to cause any pathological condition. J.F.C.

13. **The Bacteriology and Sanitation of Quick Frozen Foods.** N. H. SANDERSON, JR., Cascade Frozen Foods, Inc., Seattle, Wash. *Refrig. Engin.*, 42, No. 4: 228. 1941.

Quick frozen foods, unlike canned foods are subjected to no positive means of sterilization. The act of quick freezing only slightly reduces the number of bacteria in most of the foods frozen while prolonged storage at 0° F., although effecting a gradual reduction in the total viable cells, in no way brings about the destruction of all bacteria present. Bacterial investigations of quick frozen foods may be divided into two groups. 1. Those dealing with the effect of freezing on pathogenic bacteria which are commonly considered to be of public health significance. 2. Those dealing with the effect of quick freezing on the relatively inactive saprophytic bacteria which are normally associated with food spoilage. Emphasis is placed on careful sanitary control in the preparation of various foods for quick freezing in order to prevent the possibility of their acting as disease vendors. The author further points that methods for enumerating bacteria in frozen foods do not solve the problem of pathogenic microorganisms as possible contaminants and advocates the establishment of some standard by the frozen foods industry which will adequately cover the public health phase. Total bacteria count and *Esch. coli* count would be more desirable than total count alone.

In dealing with processing plant sanitation the processing lines should be operated on continuous flow principle free from any time consuming stoppages. Metals such as stainless steel should be employed, eliminating wood, canvas, and improperly protected metals. A plentiful supply of clean cold water for rinsing surfaces used for food preparation and handling prior to freezing is indicated, and that this cleaning be of continuous automatic nature limiting manual control to a minimum. L.M.D.

14. **Accessory Growth Factor Requirements of Some Representatives of the *Brucella* group.** STEWART A. KOSER, BEVERLY B. BRESLOVE, AND ALBERT DORFMAN, University of Chicago. *Jour. Infect. Dis.*, 69, No. 2: 114-124. 1941.

In synthetic media consisting of amino acids, glucose and inorganic salts, 7 of 8 strains of *Brucella* grew when certain accessory growth factors were added to the medium. The growth factors studied were nicotineamide, diphosphopyridine nucleotide (coenzyme I), thiamin hydrochloride (vitamin B<sub>1</sub>), diphosphothiamin (cocarboxylase), beta-alanine, calcium pantothenate, vitamin B<sub>6</sub> hydrochloride, riboflavin, inositol, glutamine, adenine, sodium

pyrophosphate and biotin. The significant accessory factors were thiamin, nicotineamide, pantothenic acid and biotin. When only thiamin and nicotineamide were added to the basic media, 4 of the 8 strains grew in serial culture, although growth was slow in some cases. The further addition of pantothenic acid accelerated growth. The addition of a biotin concentrate as a fourth accessory factor produced growth in 3 of the 4 remaining strains, but produced no marked stimulation with the strains that were able to grow without it. Other accessory factors did not substitute for these required factors and caused no greater stimulation when included with them. In these studies it was found that the optimum concentration of sodium chloride in the medium was 0.6 to 1.0 per cent. J.F.C.

## CHEESE

15. **The Control of Acid Development in Cheddar Cheesemaking.** R. M. DOLBY, Dairy Res. Inst., N. Z. New Zealand Jour. Sci. and Technol., 22, No. 4A: 289A-302A. 1940.

The effect of type of starter culture, percentage of culture, cooking temperature, "running acidity," amount of dry stirring and time of salting on rate of acid production was followed by means of pH determinations. When the percentage of starter and acidity at draining were adjusted to give the same rate of acid development in later stages of the process, cheeses of the same pH resulted. The rate of increase in acidity in the early stages was influenced by the percentage of starter and in later stages by the acidity at draining, which also controlled the acidity of the cheese. The pH of the curd at salting did not greatly affect the acidity of the cheese, but did influence the amount of salt retained and the body of the cheese. The cheese was most highly buffered between pH 4 and 5. Measurements of pH are useful in controlling acid production during cheesemaking. W.C.F.

## DISEASE

16. **Laboratory Infections Due to Brucella.** K. F. MEYER AND B. EDDIE, George Williams Hooper Foundation, University of California, San Francisco, Calif. Jour. Infect. Dis., 68, No. 1: 24-32. 1941.

From data obtained by questionnaire on 74 cases of laboratory infection occurring in the United States, the authors discuss the factors causing the greatest hazard in laboratory procedure; the frequency with which the caprine, porcine, and bovine strains, respectively, are involved; diagnostic procedures employed; and the course of the disease. Handling of cultures or specimens and inhalation of dust containing Brucella organisms appeared to cause the greatest hazards. J.F.C.

17. **A Cytophagic Reaction Employed in the Diagnosis of Brucella Infection.** MOGENS JERSILD, State Serum Institute, Copenhagen, Denmark. Jour. Infect. Dis., 68, No. 1: 16-19. 1941.

The author describes a modification of Huddleson's opsono-cytophagic test. The chief difference between the two tests is that Huddleson employed citrated blood of the patient to furnish both opsonin and phagocytes, whereas Jersild employs the patient's serum and citrated, freshly drawn blood not containing Brucella opsonin, taken from a previously tested donor. The advantages claimed are: 1. The blood specimens can be taken in the usual manner without citrate. 2. The test need not be done within 6 hours as with Huddleson's test. 3. The citrated blood is collected just prior to use, so that the greater activity of the leucocytes increases sensitivity of the test. 4. Only one control slide with the donor's blood is required for an entire series of serum tests instead of one for each patient, as with Huddleson's test.

J.F.C.

18. **Studies on the Detection of Mastitis in New Zealand Dairy Herds. I. A Field Outfit for the Bromthymol Blue Test for Mastitis.** C. M. HUME, New Zealand Dairy Board, New Zealand Jour. Sci. and Technol., 22, No. 6A: 322A-327A. 1941.

A field testing outfit for the bromthymol blue test and method of its use are described and a form for recording results is given.

W.C.F.

19. **Studies on the Detection of Mastitis in New Zealand Dairy Herds. II. Factors Influencing the Bromthymol Blue Test for Mastitis.** F. H. McDOWALL, Dairy Res. Inst., N. Z. New Zealand Jour. Sci. and Technol., 22, No. 6A: 328A-337A. 1941.

The pH values obtained electrometrically were compared with those estimated with bromthymol blue as an indicator in tests of milk of individual cows for mastitis. The colorimetric method is subject to variations or errors. The size of the sample of foremilk is important, because the pH of the milk decreases, as does the chlorine content, with successive streams of milk. Due chiefly to a loss of carbon dioxide, the pH of the milk rises on standing and also on shaking. A high fat content of the milk renders the reading of the blue color of a positive test more difficult, and a variation in the quantity of indicator affects the reading. It is recommended that a uniform technique for making the test be adopted.

W.C.F.

## FOOD VALUE OF DAIRY PRODUCTS

20. **Calcium and Phosphorus Studies in Normal People, Including Old Age.** J. DOUGLAS ROBERTSON. Lancet, 241, No. 6152: 97-100. 1941.

Blood serum studies on 60 normal people under age of 60 showed the

calcium content to be 9.9 to 11.1 mg. per 100 ml. (mean 10.393) and the phosphorus content 3.1 to 4.8 per 100 ml. (mean 3.831). In 15 healthy people aged 60 to 78 the serum calcium and phosphorus were not significantly higher than in the younger subjects. A study was made of the calcium and phosphorus balances of 9 normal people. On a calcium intake of 0.1 g. daily or on a phosphorus intake of 0.37 g. daily, all subjects were in a negative balance. For calcium the point at which equilibrium between intake and output took place was 0.45 g. daily for a 70 kg. subject. J.F.C.

## MILK

21. **Experiments on the Use of Certain Antioxidants for Control of Oxidized Flavor in Dairy Products.** W. J. CORBETT AND P. H. TRACY, Univ. Illinois. Food Res., 6, No. 5: 445. 1941.

The control of oxidized flavor in dairy products through the use of ascorbic acid, certain amino acids, concentrated water extracts from cereal flours, and a pancreatic enzyme are reported.

Tyrosine and the more soluble esters of tyrosine were found to be very effective antioxidants when used in milk in concentrations of .02 to .04 per cent. The normal amyl ester of leucine was also an effective antioxidant but imparted an objectionable off-flavor to the milk. The di-ethyl ester of glutamic acid did not produce a noticeable antioxidant effect and it gave a rather objectionable off-flavor. In copper-contaminated milk the ascorbic acid first retarded the development of the oxidized flavor and then after a certain point was reached, the development of the oxidized flavor was accelerated. In cases where 50 to 100 milligrams of ascorbic acid were added, an oxidized flavor developed in the copper-contaminated milk before all the reduced ascorbic acid disappeared.

The addition of pancreatic extract in the proportion of one part of extract to 25,000 parts of milk effectively prevented the development of an oxidized flavor. The addition of concentrated water extracts of the cereal grains was found to delay the development of an oxidized flavor in milk. The most effective product was made by drying a mixture of a water extract of a cereal flour and concentrated skim milk on a roller drier.

The addition of the various antioxidants which retarded the development of the oxidized flavor was found to have no effect on the oxidation of ascorbic acid.

The addition of the water extracts prepared from the cereal flours had only a slight antioxygenic effect when used in ice cream. A more effective product was the dried water-extract and concentrated skim-milk mixture. The addition of a commercially prepared Avenized sugar was found to give a slight antioxidant effect.

The addition to churning cream of a concentrated water extract of the

cereal flours or addition of an Avenized salt to the butter was found to retard the development of oxidized flavors in butter. P.A.D.

**22. The Phosphatase Test for Control of Efficiency of Pasteurization.**

H. D. KAY, R. ASCHAFFENBURG, AND F. K. NEAVE, Tech. Commun. 1 of Imp. Bur. Dairy Sci., pp. 54. 1939. 2 shillings.

A review.

W.C.F.

**23. A Note on the Influence of High Temperature Short Time Pasteurization on the Phosphatase Reaction and Creaming of Milk.**

W. J. WILEY, Council Sci. and Indus. Res., Australia. New Zealand Jour. Sci. and Technol., 22, No. 1A: 42A-43A. 1940.

Milk, pasteurized in a regenerative plate-type machine, with 39 seconds to reach the heating section, 29 seconds in the heating and holding sections, and 39 seconds again in the regenerator section, gave a negative phosphatase test when pasteurization was at 155° F. and a positive reaction at 153° F. Pasteurization temperatures above 155° F. reduced creaming. W.C.F.

### MISCELLANEOUS

**24. The Effect of Frozen Mass Formations on the Freezing Rate of Foods.** WM. J. FINNEGAN, Consulting Engineer, Los Angeles, Calif. Refrig. Engin. 42, No. 4: 233. 1941.

The author reports original data on tests comparing freezing rates obtained by various methods of freezing foods. The report is illustrated with diagrams, graphs, and photographs, the latter of feeding end and harvesting end of a spiral tubular freezer employed in freezing one gallon cans of orange juice. A few outstanding points in the report are listed.

1. In freezing large masses, the use of internal heat conductors will accelerate freezing rate.

2. Each food has an optimum point of final solidification which will give the highest freezing rate at a given temperature, regardless of the freezing method employed.

3. When a fluid is used as a secondary heat transferring vehicle, reversing the direction of flow at frequent intervals will increase the freezing rate and produce a more uniform product.

4. Uniform freezing may be assured by determination of the form and location of the final freezing point.

5. The point or points of "final solidification" should be selected as the point for temperature observation when arriving at a freezing rate.

6. Consideration must be given to methods of refrigeration application in arriving at freezing rate determination in conjunction with the above factors.

L.M.D.

**25. Bibliography on Refrigeration.** From the Report of A.S.R.E. Technical Committee on Agricultural Products Refrigeration under D. F. Fisher, U. S. Dept. of Agr.

1. Refrigeration of fruits and berries. H. J. Read. *Refrig. Engin.*, 39, No. 5: 303. May, 1940.
2. Cold storage locker plants spread. *American Builder*, 62, No. 6, 72-73, 102-103. June, 1940.
3. Cold storage lockers. *Nebraska Farmer*, 82, No. 6: 8. March 23, 1940.
4. Preparation of early fruits and vegetables for locker storage: Table. *Locker Patron*, 1, No. 10: 10-11. May, 1940.
5. Preservation of fruits and vegetables in refrigerated food lockers. H. L. Seaton and R. M. Griswold. East Lansing, Mich. Folded chart. Michigan State College, Extension Division. *Extension Bul.*, No. 208. 1940.
6. Fruits and vegetables for refrigerated locker plants. H. H. Plagge. *Locker Patron*, 1, No. 7: 10-11. Feb., 1940.
7. Quick-frozen foods. S. R. Winters. *Southern Agriculturist*, 70, No. 3: 46. March, 1940.
8. Cooling of milk for cheese making. Dominion of Canada, Dept. of Agr., Dairy Products Div., Marketing Service. Pub. 687, Circ. 158, 9 p. 1940.
9. Precooling tests of Indiana strawberries, cantaloupes and peaches. T. E. Hienton and K. I. Fawcett. La Fayette, Ind. *Purdue Univ. Agr. Expt. Sta. Bul.*, No. 439, 36 p. "Literature cited": p. 36. 1939.
10. Minnesota cold storage locker plants. A. A. Dowell and others. Univ. Farm, St. Paul, Minn. *Univ. of Minnesota Agr. Expt. Sta. Bul.*, 345, 39 p. 1940.
11. Refrigerated food lockers in Michigan. H. L. Seaton. In *Mich. Agr. Expt. Sta. Quarterly Bul.*, 22, No. 3: 153-159. Feb., 1940. "References" p. 159.
12. Freezing fruits and vegetables in the Southwest. J. L. Heid, *Refrig. Engin.*, 38, No. 5: 286-288. Nov., 1939.
13. Railroad service and frozen foods. Willis R. Woolrich. *Refrig. Engin.*, 38, No. 5: 277-278. Nov., 1939.
14. Farm milk house. A. J. Bell and J. M. Jensen. East Lansing, Mich. *Mich. State College Extension Division, Ext. Bul.*, 206, 11 p. 1940.
15. Dry-ice as a transport refrigerant. N. E. MacLean. *Ice and Refrig.*, 97, No. 5: 330. Nov., 1939.
16. Polar chest locker system. E. C. Lloyd. *Refrig. Engin.*, 38, No. 5: 308-309. Nov., 1939.

L.M.D.



# JOURNAL OF DAIRY SCIENCE

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# ABSTRACTS OF LITERATURE

## ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

### 26. **The Significance of Tannic Substances and Theobromine in Chocolate Milk.** W. S. MUELLER, Massachusetts State College, Amherst, Mass.

The relative toxicity of pure theobromine, pure tannic acid and two cocoa powders varying in tannic substances content was determined by feeding these substances in a basal diet to white rats. Theobromine was non-toxic to albino rats when the ration contained 0.27 per cent of this alkaloid, and tannic acid was toxic when the ration contained 2 per cent of this substance. A cocoa powder containing 12.15 per cent of tannic substances was more toxic than a cocoa powder containing only 2.67 per cent of tannic substances, but was less toxic than pure crystalline tannic acid. A concentrated extract of cocoa was non-toxic to rats when the ration contained 8 per cent. The hemoglobin levels of the blood of rats fed theobromine, crystalline tannic acid, and cocoa powder containing varying amounts of tannic substances did not vary from the normal enough to be of any significance. Results from this study indicate that the toxicity from cocoa can be greatly reduced by selecting a cocoa or chocolate which is low in tannic substances, or preferably using an extract of cocoa as the flavoring material when feasible.

### 27. **The Advantage of Grinding Atlas Sorghum Grain for Dairy Cows.** F. W. ATKESON AND G. H. BECK. Contribution No. 140, Department of Dairy Husbandry, Kansas State College, Manhattan.

Feeding trials with dairy cows were conducted to determine the value of grinding of Atlas sorgho grain as measured by the amount of grain recovered in the feces. The sorgho grain was fed as whole grain representing a fineness modulus of about 4.5; as coarsely ground grain representing a fineness modulus of 3.65; and as finely ground grain of a fineness modulus of 2.44. A burr mill was used for the coarse grinding and a hammer mill for the fine grinding.

The cows were fed through three ten-day periods using in the first period whole grain, in the second coarse ground or cracked grain, and in the third finely ground grain. All feces voided during the last three days of each ten-day period were collected and the grain recovered.

The trial lots included cows receiving only Atlas sorgho grain and alfalfa; a comparison of the degree of utilization of sorgho grain by cows on a balanced ration and cows on a deficient ration; and the effect on utilization by cows receiving the sorgho grain in silage as compared to sorgho grain fed as a concentrate.

The results of all three groups were in accord with each other in that feeding whole grain resulted in excessive waste while coarse grinding was more satisfactory than fine grinding considering cost of grinding and consistency of feed, and both grindings decreased the waste remarkably. The first lot receiving alfalfa and sorgo grain resulted in a recovery in the feces of 42.0 per cent of the grain when fed whole; 4.8 per cent when the grain was coarsely ground and 1.5 per cent when finely ground. In the group comparing deficient rations on utilization of sorgo grain the recovery results were in accord with the first group, but cows receiving deficient rations averaged a grain waste of five times greater than cows receiving normal rations. The trial group fed whole sorgo grain in silage showed a grain recovery in the feces of 10.7 per cent. However, the silage fed was immature, the grain content as fed being 1.3 per cent.

## BACTERIOLOGY

28. Heat Resistant and Heat-Loving Bacteria. The Problem—Their Control. W. B. SARLES, Univ. Wisconsin. Milk Plant Monthly, 30, No. 8: 3, 33, 42. 1941.

Heat-resistant and heat-loving bacteria may be classified into several main groups. Their presence in milk is undesirable because of subsequent spoilage and high counts in the pasteurized products. Improperly treated utensils and coat of the cow are the two chief sources of contamination. Control of thermophilic bacteria within the plant involves scrupulous cleaning and sterilization of all equipment; short runs with vat pasteurizers; use of air space heaters; avoiding repasteurization; and possibly employment of hot-short pasteurization. The methylene blue test at 122° to 125° F. may be used to detect thermophiles. G.M.T.

29. Control of Heat Resistant and Heat-Loving Bacteria. W. B. SARLES, Univ. Wisconsin, Madison. Natl. Butter and Cheese Jour., 32, No. 10: 18. 1941.

For abstract see preceding abstract.

W.V.P.

30. Behavior of Microorganisms at Subfreezing Temperatures. III. Influence of Sucrose and Hydrogen-Ion Concentrations. V. H. McFARLAND, Bur. Agr. Chem. and Engin., U.S.D.A., Washington, D. C. Food Res., 6, No. 5: 481. September–October, 1941.

Cells of a cold tolerant *Saccharomyces* and of *Esch. coli* were suspended in sucrose solutions varying from 1 to 50 per cent. Viability studies were made at pH 6.5, 5.0 and 3.6 to 3.7 when held at -10° C. and at -20° C.

It was found that viability did not always correlate with sucrose concentration except at the low pH level. High concentrations of sucrose

tended to retard destruction of the yeast cells although greater destruction occurred in the medium concentrations than in distilled water. On the other hand, *Esch. coli* was destroyed to a greater extent in distilled water than in any of the sucrose concentrations.

When pH was the only variable, the low pH levels (3.6–3.7) proved to be more destructive to both microorganisms than the higher levels. When temperature was the only variable greater kills were obtained at  $-10^{\circ}\text{C}$ . than at  $-20^{\circ}\text{C}$ . F.J.D.

## BUTTER

31. **Old and New Facts about Churns and Churning.** F. H. ABBOTT, Univ. Calif., Davis. Natl. Butter and Cheese Jour., 32, No. 11: 12. 1941.

Round fat globules are kept apart in milk and cream by the viscosity of the suspending medium, by an adsorbed layer of nitrogenous milk solids and lactose and by the negative charge on the fat globules. Agitation ruptures the adsorbed layer, the fat globules adhere to each other and when the butter "breaks" there is present a skimmilk-in-fat emulsion. New types of roll-less churns are more sanitary; metal churns can be sterilized with hot water or steam and butter made in them rarely shows yeast or mold. W.V.P.

32. **A Study of Some Factors Influencing the Phosphatase Reaction of Flash Pasteurized Cream and Butter Made from It.** W. J. WILEY, F. S. J. NEWMAN, AND H. R. WHITEHEAD, Council Sci. Indus. Res., Victorian Dept. Agr. and Dairy Res. Inst. of N. Z., resp. Jour. Council Sci. Indus. Res., Australia, 14, No. 2: 121–128. 1941.

When the Kay and Graham "long" phosphatase test was used on cream pasteurized in the vacreator and on serum from butter made therefrom, the butter gave much higher values than the cream from which it had been made. Addition of salt, galactose or sucrose to cream (or butter) produced no immediate effect on the test, but after several hours phosphatase values were considerably higher. Vacuum drying the cream at  $40^{\circ}\text{C}$ . caused a similar increase, and even storage of the flash pasteurized cream led to a slow increase in phosphatase values. The authors theorize that there apparently is "binding of a small and varying proportion of the enzyme in the cream in such a way that it escapes destruction during a very short heat treatment." It is supposed that salt, sugars and drying cause the release of this "bound" phosphatase. Tests on cream flash pasteurized in the laboratory gave similar results.

There was no evidence of the production of phenolic substances in the cream by bacteria. Plate counts showed that there was more destruction

of bacteria by the vacreator method than by the usual holding method, although lower phosphatase values were obtained from cream subjected to the holding method.

The authors conclude that "the phosphatase test therefore cannot be used on butter as a means of checking whether the initial cream has been properly flash pasteurized."

W.C.F.

## CHEESE

33. **Pioneer Days in Cheese Making.** E. L. ADERHOLD, Natl. Butter and Cheese Jour., 32, No. 11: 16. 1941.

**The Good (?) Old Days.** A. T. BRUHN, Wisconsin Dept. Agr., Madison, Wis. Natl. Butter and Cheese Jour., 32, No. 11: 22. 1941.

These two articles review the changes in the cheese industry, relative to the methods of payment for milk, starters, boards of trade, styles of cheese, buildings and equipment, boxes, utensils and inspection.

W.V.P.

34. **Bacteriology of Cheese. VI. Relationship of Fat Hydrolysis to the Ripening of Cheddar Cheese.** C. B. LANE AND B. W. HAMMER. Iowa Agr. Expt. Sta. Res. Bul., 291. 1941.

The studies were carried out with four types of milk, (1) raw milk, (2) pasteurized milk, (3) skim milk (raw or pasteurized) plus homogenized cream (raw or pasteurized) and (4) pasteurized material containing lipolytic enzyme.

In some trials cheese made from raw homogenized cream plus raw or pasteurized skim milk was more satisfactory than that made from raw milk. Although cheese from the raw homogenized cream plus raw or pasteurized skim milk commonly was characterized by a rancid flavor early in the ripening period, this rancidity tended to disappear, and when well ripened, the cheese was superior in flavor to that made from pasteurized milk or pasteurized homogenized cream plus pasteurized skim milk.

Addition of pancreatin to pasteurized milk resulted in an objectionable rancid flavor and in the ripened cheese this was in many cases accompanied by a pronounced bitter flavor.

The titratable acidities of cream-sugar mixtures increased when desiccated mammary tissues or liquid extracts of them were added and the mixtures incubated at 37° C. for 3 and 7 days. Tissue extracts produced less fat hydrolysis than the corresponding desiccated tissue.

Addition of desiccated mammary tissues or their extracts to pasteurized milk resulted in an increase in fat acidity and a slight increase in soluble nitrogen in the cheese. The flavor of cheese made from pasteurized milk was improved by addition of mammary tissue or its extract. In some trials

when spleen tissue or its extract were added to pasteurized milk, the flavor of the cheese was improved. Little improvement in flavor of cheese resulted when liver extracts were added to pasteurized milk. P.R.E.

**35. Cottage Cheese—a Healthful Food for the Lenten Season and the Year Round.** H. A. RUEHE, Univ. Illinois, Urbana, Ill. *Milk Plant Monthly*, 30, No. 3: 27-28. 1941.

The market possibilities of cottage cheese together with methods of manufacture and causes of defects are presented in detail. The possible uses of cottage cheese include: appetizers, soups, salads, main dishes and sandwich fillings. The size and characteristics of cottage cheese curd may be varied to suit the consumer demand. Ten steps are given in making a flaky curd cottage cheese. Tough and rubbery curd is caused by: heating the curd too high or holding at the cooking temperature too long; cutting the coagulum before sufficient acidity has developed; and not using a sufficient amount of rennet extract. A soft, pasty curd is caused by: permitting too much acid development; using too much rennet extract; too low a cooking temperature or insufficient cooking time; and too high a pasteurization temperature. G.M.T.

**36. Danger Confronts the Dairy Industry.** RALPH AMMON, Director Wis. Dept. Agr., Madison. *Natl. Butter and Cheese Jour.*, 32, No. 11: 66. 1941.

Dangers listed are: possibility of over-expanded production after the current crisis; disorderly diversion; unfair legislation, particularly that pertaining to oleo; war-hysterical appeal for use of substitutes for butter; and regimentation and control of industry by Federal authorities. Suggestions to protect the industry are: co-operation in production for defense; co-operation between states to avoid adjustment shocks after crisis; fight for a fair deal by demanding repeal of oleo standard, demanding laws prohibiting fraudulent advertising of oleo in semblance of butter; abolishment of the Consumer Counsel Division of the U.S.D.A.; manufacture and advertising of highest quality butter; and the preservation of democracy by resistance to bureaucratic control and political subjugation. W.V.P.

**37. Leadership.** C. R. BARKER. *Natl. Butter and Cheese Jour.*, 32, No. 10: 14. 1941.

Leadership can be established in the cheese business on a quality rather than a volume-of-business basis. This must be done through intelligent sales efforts with emphasis on excellence, nutritive value and recognition of the responsibility of the manufacturer to the public. W.V.P.

## CHEMISTRY

38. **Composition of the Milk of the Monkey.** G. VAN WAGENEN, H. E. HIMWICH, AND H. R. CATCHPOLE, Dept. Obstetrics and Gynecology, the Adolescence Study Unit, Yale Univ. Soc. Expt. Biol. and Med. Proc., 48: 133. 1941.

Average values for 9 animals gave: protein 2.1 per cent; carbohydrate 5.9 per cent; fat 3.9 per cent; and ash 0.26 per cent. It is pointed out that in composition monkey's milk is similar to that of the human being and differs from cow's milk in having a lower percentage of protein and ash and a higher percentage of milk sugar. Milk samples were obtained between the 35th and 103rd day of lactation and no systematic changes were observed during this interval in the percentages of the principal milk constituents or in the calorific values ascribable to them. R.P.R.

39. **Quantitative Determination of Dissolved Oxygen. Ascorbic Acid Oxidase Method.** PAUL F. SHARP, DAVID B. HAND, AND E. S. GUTHRIE, Cornell Univ., Ithaca, N. Y. Indus. and Engin. Chem., Analyt. Ed., 13, No. 9: 593. 1941.

This new method for the determination of the dissolved oxygen of fluids containing organic matter was developed especially for making determinations on milk. The determination was made several thousand times on milk, water, buffer solutions and bacteriological media, it being possible for one person to make 30 determinations in a half day. Since fresh milk contains only about  $1/3$  enough ascorbic acid to react with the dissolved oxygen, additional ascorbic acid is first added to each sample. The total ascorbic acid content of the milk is then determined by titration. A quantity of ascorbic acid oxidase which acts as a catalyst is added to the milk and the sample is allowed to stand 15 minutes during which time the enzyme causes all the dissolved oxygen to react with the reduced ascorbic acid. The enzyme is then inactivated by sulfuric acid and the ascorbic acid content of the sample is again determined by titration. The dissolved oxygen content of the sample is obtained by a simple calculation. Complete directions for preparing reagents and apparatus are given. A specially designed oxygen analysis tube which avoids transference of the liquid and protects it from air is used for the determinations. Ascorbic acid oxidase is prepared from the juice of green cucumbers. The method shows good agreement on water when compared with the Winkler method. The probable error is 0.1 to 0.2 part per million. B.H.W.

40. **Instrumental Methods of Chemical Analysis.** RALPH HOLCOMBE MÜLLER, Dept. Chemistry, New York Univ., Washington Square,

New York, N. Y. Indus. and Engin. Chem., Analyt. Ed., 13, No. 10: 667. 1941.

This paper comprises the entire issue of this Journal. The use of instruments in analysis and testing is discussed and descriptions and illustrations of all the more important types of instruments used in chemical analysis are presented.

B.H.W.

- 41. Polarographic Determination of Ascorbic Acid.** MARY MANN KIRK, N. Y. State Agr. Expt. Sta., Geneva, N. Y. Indus. and Engin. Chem., Analyt. Ed., 13, No. 9: 625. 1941.

The author reports the results of preliminary work on the use of the polarograph for the determination of ascorbic acid. No quantitative determinations were run but results were obtained which indicated that the method could be adapted for accurate quantitative analysis by comparing curves obtained when an unknown solution was used, with calibration curves.

B.H.W.

- 42. The Colorimetric Determination of Lactic Acid in Biological Material.** S. B. BARKER AND WILLIAM H. SUMMERSON, New York Hospital and Depts. of Medicine and Biochemistry, Cornell Univ. Medical College, New York City. Jour. Biol. Chem., 138, No. 2: 535. 1941.

A method for the colorimetric determination of lactic acid in biological material is described in which the lactic acid is converted into acetaldehyde by treatment with concentrated sulfuric acid and the acetaldehyde determined by its color reaction with parahydroxydiphenyl in the presence of cupric ions. The color is read in a photoelectric colorimeter with a filter having a peak transmission at 560 m $\mu$ .

V.C.S.

- 43. The Chemical Determination of Nicotinic Acid in Milk and Milk Derivatives.** C. I. NOLL AND O. G. JENSEN, Borden Co. Res. Div., Biological and Chemical Labs., Bainbridge, N. Y. Jour. Biol. Chem., 140, No. 3: 755. 1941.

The method of Melnick and Field appears to be well adapted to the estimation of nicotinic acid in milk. A discussion of the limitations of chemical methods for the determination of nicotinic acid, in respect to specificity of the reactions, the use of an appropriate blank, and the interference of colored solutions, is presented.

The nicotinic acid values reported for a group of dry skim milk samples, as determined by the method presented, varied from 14 to 28 gamma per gram (average 18.3) and are lower than most values reported in the literature.

V.C.S.



of Irish Moss about 50 per cent, irrespective of the level included in the diet. In general the animals grew very well except those receiving a diet containing 20 per cent Irish Moss, in which group there was a 50 per cent mortality. A progressive depression in the apparent digestibility of dry matter of the remainder of the diet was noted as the level of agar or Irish Moss was increased. This amounted to almost 10 per cent at the higher levels.

F.J.D.

51. **The Presence of Free and Combined Thiamine in Milk.** NELLIE HALLIDAY AND HARRY J. DEUEL, JR., Dept. Biochemistry, Univ. Southern California School of Medicine, Los Angeles. Jour. Biol. Chem., 140, No. 2: 555. 1941.

The thiamine content of Certified Holstein milk was found to average 23.4 and 40.5 mg. respectively for the free and total thiamine. Only approximately 60 per cent of the thiamine in milk is present in a free state, the rest being broken down with phosphatase or with taka-diastase but only when a proteolytic enzyme such as papain is also used.

Methods for the determination of free and combined thiamine in milk are given.

V.C.S.

## ICE CREAM

52. **Outline of Activities for the Year 1941.** ROBERT C. HIBBEN. Special Bulletin, Internat. Assoc. Ice Cream Mfrs., Washington, D. C. November, 1941.

The association has cooperated with other food industries in getting preference ratings for materials for replacement and repairs of equipment. Work has been continued with the Federal Food and Drug division regarding the definitions and standards of identity for frozen desserts to be adopted under the Food, Drug and Cosmetic Act of 1937. Federal and state legislation affecting the ice cream industry has been followed, and such matters as the Federal Tax Bill, price control legislation, labor measures, highway and trucking bills, etc., have been studied.

Consumer education has received attention, and several booklets for consumers have been released. The association took an active part in the promotion of June Dairy Month. Inter-industry contacts have been maintained with a number of industrial groups and committees.

The statistical and accounting bureau of the I.A.I.C.M. published a new accounting system which has already been installed by a large number of ice cream manufacturers. The bureau has made ice cream "expense comparisons" and has made an analysis of equipment used by the industry. The sales index of ice cream for 1941 has also been compiled.

The activities of the Ice Cream Merchandising Institute are also included in the bulletin. Attendance at the 1941 Merchandising Short Courses was approximately 5,000 and plans are completed for the 1942 courses. There are many other activities of the institute, such as the publication of monthly booklets on selling ice cream, the preparation of monthly window poster service kits, and so on.

M.J.M.

**53. Ice Cream Sales Index.** Special Bulletin. Statistical and Accounting Bureau, Internat. Assoc. Ice Cream Mfrs., Washington, D. C. December, 1941.

This bulletin contains an analysis of ice cream sales for 1941, compared with 1940. The sales are for the period of January 1 through August 31. For this period of 8 months, sales in the United States increased 18.3 per cent over the like period in 1940. In Canada the increase for the same period was 36.36 per cent, and for the Territories of the United States the increase was 52.46 per cent.

Every state in the United States reported increased sales for the first 8 months of 1941, with increases of more than 20 per cent for 14 states. The sales increase was greatest in the Southern states (24.9 per cent) and least in the Western states (14.2 per cent).

A supplement to the bulletin contains the preliminary production report for 1940 as compiled by the Agricultural Marketing Service. The total production of ice cream for 1940 is given as 316,236,000 gallons, which is an increase of 13 million gallons over 1939. The per capita production in quarts is given by states. In the District of Columbia the highest annual per capita production of 24.24 quarts was reported. The percentage of the total U. S. production is also given by states for the year 1940. Pennsylvania led with 13.29 per cent of the total production, New York was second with 12.5 per cent, and Illinois was third with 6.84 per cent of the total.

M.J.M.

**54. Our New Army as a Market for Ice Cream.** VINCENT M. RABUFFO. Ice Cream Trade Jour., 37, No. 8: 6. 1941.

A survey was conducted by the author to obtain figures on the consumption of ice cream by the soldiers in the many army camps throughout the nation. The figures obtained showed that 565,000 men out of an estimated total of 1,448,600 consumed approximately 107,000 gallons of ice cream during the month of May. On this basis it was estimated that this amount would be equivalent to a yearly per capita consumption of 3.8 gallons which is considerably above the national average. The prices paid by the army for standard brands of ice cream compared very favorably with that paid by ice cream dealers. The army recognizes the wholesomeness of ice cream

and serves it from one to three times a week in the mess halls and in addition large quantities are consumed in the canteens, post exchanges and clubs. This new business has raised the value of products manufactured in states where large numbers of men are in army camps. W.H.M.

**55. What is Happening to Product Costs.** VINCENT M. RABUFFO. *Ice Cream Trade Jour.*, 37, No. 8: 10. 1941.

The cost of ingredients used in the manufacture of ice cream has increased about 20 per cent during the past year and has resulted in increasing the cost of manufacturing a gallon of ice cream from 10 to 16 cents per gallon. Labor and overhead costs have also gone up and as a result ice cream prices have generally advanced throughout the nation. In some instances price advances have not kept pace with increased costs, and in other cases manufacturers have been forced to reduce the fat content of their ice cream and amount sold in various packages and novelties to partially offset increased costs. In many instances retailers have increased the selling price of ice cream and items containing ice cream far more than the percentage increase in the cost of their product. If sales of ice cream are to continue at a satisfactory volume, close cooperation between the manufacturer and retailer will be necessary. W.H.M.

**56. No Place Like Home for Greater Sales.** OWEN M. RICHARDS. *Ice Cream Trade Jour.*, 37, No. 8: 14. August, 1941.

A survey conducted by the American Dairy Association in some of the large cities reveals that ice cream ranks first as a dessert in public eating places and in the home when guests are present, but it ranks far down the list at regular meal time in the home. Families with children and families of high and middle incomes had ice cream delivered to the home more frequently than those in the lower classifications and those without children. Most ice cream was purchased through drug stores although the percentage varied in different cities. W.H.M.

**57. The Homogenization of Ice Cream Mix.** B. I. MASUROVSKY. *Ice Cream Trade Jour.*, 37, No. 8: 22. 1941.

Faulty homogenization can be determined by diluting the homogenized ice cream mix with two parts of water to one part of mix and allow it to stand in a tall test tube at 50° F. for two or three hours. The degree of stratification of layers of ice cream and their density serves as an index of homogenization efficiency.

A new method of measuring homogenization efficiency has been worked out by the laboratory of the Creamery Package Manufacturing Company, Chicago, Illinois, which consists of microscopic examination of diluted prod-

ucts and a classification of the globules according to the size of the globules. From these figures an homogenization index can be calculated.

W.H.M.

- 58. Our New Army.** LT. COL. PAUL P. LOGAN, Office of Quartermaster Corps, Chief, Subsistence Branch. *Ice Cream Trade Jour.*, 37, No. 9: 10. 1941.

This article explains how ice cream is bought for use by the U. S. armed forces in its mess halls, post exchanges, and service clubs. The use of ice cream on the menu is left to the discretion of the officers in charge of each organization mess and the frequency of its use depends largely upon the taste of the men comprised in the mess. The ice cream for this purpose is purchased on definite quantity contracts entered into on the basis of competitive bids. The contracts are usually made on monthly basis, but the Corps Area Commander may authorize contracts covering a period of three months.

Each of the 147 Army posts and camps in the continental United States has one or more post exchanges. The army furnishes the building and equipment for these exchanges. In buying ice cream the PX officers are not required to make any contract for a period of time specified by the army or for any regulatory quantity of ice cream. This is left entirely up to the exchange in each individual case.

Recreational centers operated by the affiliated groups of the U.S.O. are separate from the army camp but each of these will have a fountain. Each will purchase its ice cream in a way similar to that used by the PX officers.

W.H.M.

- 59. A Method of Testing Flavors.** B. I. MASUROVSKY, Research Editor. *Ice Cream Trade Jour.*, 37, No. 9: 27. 1941.

An economical and time-saving method of testing ice cream flavor and color is described. It consists of placing 8 to 16 oz. of ice cream mix, carrying the flavor to be tested in an aluminum container which has a syphon arrangement leading to the head where provision is made to connect a cartridge filled with nitrous oxide under pressure. The mix is charged with gas, shaken and placed in a quart container and hardened with dry ice. Colors can be tested in the same way. A table showing the amount of flavor required for 5 gallons of ice cream mix is presented.

W.H.M.

- 60. Production Costs Can Be Lowered.** PAUL VASTERLING, Dufold Sales Co., Racine, Wis. *Ice Cream Trade Jour.*, 37, No. 10: 32. 1941.

The ice cream manufacturer who may want to reduce the ingredient cost of his cream will be interested in this article in which the author suggests

increasing the serum solid content of the ice cream, thus making it possible to take a higher yield without any sacrifice in the weight of the finished product. W.H.M.

61. **Equipping a Dairy Products Control Laboratory.** PAUL H. TRACY, University of Illinois, Urbana. *Ice Cream Trade Jour.*, 37, No. 10: 62. 1941.

The author has listed the equipment necessary for a bacteriological control laboratory for a dairy products plant, and estimates that it will cost approximately \$1000 to purchase and install the equipment. W.H.M.

62. **Controlled Retail Stores,** ANONYMOUS. *Ice Cream Field*, 38, No. 4: 26. October, 1941.

It is stated that invariably merchandising conferences stress (1) store clean up; (2) display and advertising, and; (3) handling merchandise properly. It is claimed that such programs always meet with the following obstacles: (1) dealer resents implication that his store is in bad condition; (2) he fails to execute program as outlined at the conference, and; (3) he permits competitive lines to occupy all available space.

Several specific cases are cited in which ice cream sales had materially increased as a result of controlled retail stores. Although some manufacturers have failed in operating their own stores, it is pointed out that the failure is due largely to lack of properly controlled retail stores.

It is concluded that a well-designed and properly operated plant store or town store will serve as an effective permanent advertisement. It is also claimed that such stores when properly operated are not in competition with dealers but serve as proof of effective means of getting business. Develop your own distinctive merchandising program rather than match "competitive schemes" is the advice given. W.C.C.

63. **Your Delivery Problem.** B. P. FORTNEY, Warnsman-Fortney Body Co. *Ice Cream Field*, 38, No. 4: 27. October, 1941.

It is stated that the operating costs of old bodies and chassis are often more than the depreciation and operating costs of new trucks and bodies, in addition the latter maintain more uniform temperature and are more convenient.

Good-looking modern trucks painted in pleasing colors with attractive sign work serve as effective advertising.

It is stated that the principal changes for 1942 will be those necessitated by substitution materials, such as aluminum and corkboard, occasioned by the defense program. The author recommends the purchase of refrigerator trucks from well-established manufacturers. W.C.C.

- 64. Now You Can Sell Peach Ice Cream.** JAMES S. LAWLESS, Frosted Fruit Products, Los Angeles. *Ice Cream Field*, 38, No. 4: 6. October, 1941.

Peach ice cream has been a disappointment to manufacturer, retailer, and consumer, it is stated, because the pleasing flavor of the peach itself is elusive. This is evident from the fact that in 1938 peach ice cream accounted for only 1.46 per cent of the national ice cream production.

The author points out that the nectarine, in reality a variety of peach, is superior to ordinary peach as a source of flavor for ice cream. The nectarine can be processed by quick-freezing methods and used satisfactorily throughout the year as a source of flavor in ice cream or as fruit sundae topping. For such purposes tree ripe fruit is essential. It should be pre-cooled, pureed without air incorporation, packed with about 25 per cent sugar, and then frozen rapidly. W.C.C.

- 65. Food Value of Ice Cream.** J. H. FRANDSEN, Mass. State College. *Ice Cream Field*, 38, No. 4: 13. 1941.

**Ice Cream: A Food—Not a Fat.** T. R. FREEMAN, Univ. Florida. *Ice Cream Field*, 38, No. 4: 10. 1941.

**Ice Cream and Better Nutrition.** J. HOFFMAN ERB, Ohio State Univ. *Ice Cream Field*, 38, No. 4: 10. 1941.

**Ice Cream and National Emergency.** W. C. COLE, Univ. California. *Ice Cream Field*, 38, No. 4: 10. 1941.

**Ice Cream Food Value Is Important in Advertising.** P. S. LUCAS, Michigan State College. *Ice Cream Field*, 38, No. 4: 10. 1941.

This series of articles on the food value of ice cream brings out the following points:

1. Ice cream is an important food source of calcium, phosphorus and the vitamins.

2. Ice cream can furnish a part of the weight increasing, or reducing diet. When consumed in addition to the regular diet ice cream increases the caloric intake; or when used to replace rich desserts of high caloric value the net caloric intake is accordingly reduced.

3. The caloric value of one quart of vanilla ice cream is compared with several other foods in terms of the amount of each required to furnish the same amount of energy.

4. The proportions of the adult nutritive requirements supplied by a 3½ oz. serving of ice cream and an 8 oz. glass of milk are given in tabular form.

5. It is pointed out that ice cream contains about 4 times the calories, slightly more mineral and serum solids and 3 times as much vitamins A and D as an equal weight of fluid milk.

6. The place of ice cream along with other dairy products in a national nutrition program is stressed.

7. Ice cream can be effectively advertised on the basis of its nutritive value. W.C.C.

66. **Putting False Faces on Formulas.** W. R. VAN METER, Kingston Cake Co. Food Indus., No. 10: 41-42. 1941.

You can disguise a valuable formula so that competitors cannot steal it by using arbitrary units of measurements and adopting coined names for ingredients. In the dairy and baking industry this appears to have merit as the great turnover in employment affords opportunity to carry procedures from one company to another. J.C.M.

67. **New Quick Freezer Tests Foods in South.** EARLE MAUDLIN. Food Indus., No. 10: 46-47. 1941.

This article deals with the experiments in freezing fruits, berries, and poultry. The experiments were conducted in five states by the T.V.A. and Georgia Experiment Stations. Much of value to the ice cream industry is contained in this article. J.C.M.

68. **Dehydration Looks Up.** L. V. BURTON, Editor Food Indus. Food Indus., No. 9: 53-55. 1941.

For centuries man has dehydrated meats, fish and fruits with success, but never have they had any success with dehydrated vegetables until a few years ago.

The idea came from an experience frozen vegetable companies had, *i.e.*, that you must inactivate the enzymes or else the food will change flavor. Inactivation of enzymes is also a factor in preparing fruits for use by the ice cream industry. J.C.M.

## MILK

69. **Refrigeration Equipment in Dairies.** H. O. ROBERTS, JR., Central Power and Light Co., Corpus Christi, Texas. Refrig. Engin., 42, No. 5: 305. 1941.

Control of bacteria by methods of refrigeration as practiced by Texas dairymen. These methods include mechanical refrigeration by means of wet storage and dry storage applications, the latter frequently employing brine circulation outside the dry storage room for precooling milk. Manufactured ice is used where electric service is not available, and it is used by producer-retailers for icing bottled milk for delivery. It is stated that under Texas conditions 0.98 kw. hr. of electricity are required per 100 lb. milk cooled, and that where electric service is available, most dairies are using some form of mechanical refrigeration. The author states that the large

wholesale dairies largely use direct expansion coolers in combination with the insulated tank. For dry storage of bottled milk among the larger distributors the unit blower-cooler is noted along with the older direct expansion coil system. L.M.D.

**70. The Use of Flavored Milk Drinks in the Dairy Industry.** GIDEON HADARY, Madison, Wis. *Milk Plant Monthly*, 30, No. 1: 29-31. 1941.

Chocolate milk is the outstanding flavored milk drink despite various sporadic attempts to sell fruit-flavored milk. The ratio of chocolate to plain milk consumed by high school students was found to be 2 to 1, whereas in junior college the ratio was about 50-50. As children grow older the tendency is to drink less milk. Industrial workers prefer chocolate milk. To overcome industrial fatigue many industrial plants are introducing milk service which promises to be a potential outlet for milk sales. Little has been done in this country toward promoting fruit-flavored milk, a product which has enjoyed widespread popularity in England. States vary widely in their legal standards and definitions for chocolate milk. G.M.T.

**71. Improving the Quality of Milk Supplies in Small Communities.** C. J. BABCOCK, U. S. Dept. Agr., Washington, D. C. *Milk Plant Monthly*, 30, No. 2: 28-30. 1941.

A committee report. Lack of realization of necessity of adequate sanitary control of the milk supply as well as proper pasteurization, coupled with cost of milk inspection are believed to be the factors chiefly responsible for a lower quality milk supply in the smaller communities. Pasteurization has failed in many small communities because oxidized flavor, caused by copper contamination is considered typical of pasteurized milk. The cost of sanitary control in the average municipality having local control in 1936 was reported by U. S. Public Health Service to average 7.1 cents per capita and ranging from 8 cents in the smallest municipality to 5 cents in the largest. Apparently to secure satisfactory milk supply in a small community, consciousness to the need of improving the milk supply must be awakened and some method must be devised to keep the cost of adequate milk control from being excessive. The former must necessarily be accomplished through educational means whereas the area plan of milk control and leadership supplied by the state control officials offer a possible solution to the latter. G.M.T.

**72. Homogenized Milk Is Here to Stay and Is Well Past the Experimental Stage.** L. K. CROWE, Univ. Nebraska, Lincoln, Neb. *Milk Plant Monthly*, 30, No. 2: 36, 38. 1941.

Highlights in research on homogenized milk and their importance



to commercial practice are presented. Homogenization lowers protein stability; lightens the color of milk; lessens the occurrence of oxidation; induces development of rancidity in raw milk; increased sedimentation; makes fat testing by accepted Babcock procedure difficult; breaks up bacterial clumps; makes utilization of returned milk a special problem. Homogenized milk must always be pasteurized with no lag of time between homogenization and pasteurization to prevent rancidity. The problem of sedimentation is overcome by clarification. Returns may be used in ice cream mix, chocolate milk, standardizing milk for cream or for whole milk cheese, cottage cheese, and for standardizing cream. Thermophilic bacteria may be a problem when returns are used. G.M.T.

**73. Factors Influencing the Flavor of Milk.** PAUL F. SHARP, Cornell Univ., Ithaca, N. Y. *Milk Plant Monthly*, 30, No. 2: 31-34. 1941.

Flavor, involving both odor and taste, is a very important commercial and dietetic property of milk, assuming a greater role each year. The author names many of the more current off-flavors in milk and suggests that the group can be classified on the basis of the following causes: 1. microbial growth and decomposition; 2. feed; 3. absorbed; 4. chemical composition of the milk; 5. processing and handling; 6. enzymatic and catalytic.

Each group is discussed separately and general remedies for its elimination or prevention in milk are given. The paper is an excellent, brief, survey of milk flavors and a current review of some of the trends in recent research in that field. G.M.T.

**74. Homogenization of Milk by Sonic Vibration.** EDWIN P. BROWN, Mgr. Loudon Hills Farms, South Montrose, Pa. *Milk Plant Monthly*, 30, No. 3: 52. 1941.

Commercial experience with a sonic oscillator for homogenizing milk was favorably reported. Laboratory findings on about 100 samples revealed bacterial counts ranging from 0 to 200 per ml. The highest curd tension was 10 grams, the lowest 5 grams, and the average for the period under study was 8.5 grams. Although stabilization of emulsion was reasonably satisfactory, extremes possible in pressure homogenization were not accomplished. It was claimed that in sonic homogenization, clarification was unnecessary. G.M.T.

**75. The Physical Structure of Milk.** E. L. JACK, Univ. California. *Milk Plant Monthly*, 30, No. 3: 25, 26. 1941.

The various constituents of milk are discussed from the standpoint of their size and physical relationship to each other. Despite the relatively

high water content milk is a liquid because of the size and shape of its particles. The percentage solids of many common solid foodstuffs relatively high in water are given. The properties and conditions of the constituents affecting their action in milk such as wheying and creaming are given.

G.M.T.

- 76. Cereal Milk.** WAYNE H. BABCOCK, Babcock Dairy Co., Toledo, Ohio, *Milk Plant Monthly*, 30, No. 4: 1941.

Cereal milk, known also as cereal cream, 10 per cent, half and half, breakfast cream and by many trade names, contains approximately 10 per cent fat and may or may not have 1 per cent of S.N.F. added in the form of fresh condensed skim milk or skim milk powder. The product is pasteurized at 150° F. and homogenized at pressure ranging from 1500 to 2000 pounds. Milk intended for cereal milk was purchased at class II or cream price. Since its introduction in Toledo in January, 1939, the average equivalent pint unit daily sales have increased from 166 to 4,584 in November, 1940, with only a three per cent drop in cream sales, which could not be attributed alone to the sales of cereal milk. Whether sales of cereal milk has hurt milk sales is debatable.

G.M.T.

- 77. The Value of Laboratory Control.** D. E. NORSINGER, Richmond Dairy Co., Richmond, Va. *Milk Plant Monthly*, 30, No. 5: 50-52, 70. 1941.

A plant laboratory is an aid to the manufacturing departments of a plant in producing quality and preventing waste, as well as to the sales and advertising departments. Much information concerning the product may be obtained through laboratory control. Among possible tests are those on butterfat, acidity, uniformity, keeping quality, solids-not-fat, quality of raw material, sanitation, microscopic and standard plate count, phosphatase, organoleptic, and microscopic observation of homogenized products. Proper equipment and an adequately trained man with experience are essential for satisfactory laboratory control.

G.M.T.

- 78. Milk and Men.** J. C. NISBET, Ohio Dairy Products Assoc. *Milk Plant Monthly*, 30, No. 5: 66-69. 1941.

The requirements of the human body and the part played by milk in human nutrition are discussed and somewhat dramatized. Comparisons between calcium content of milk and other foods are given.

G.M.T.

- 79. Some Factors Affecting Wheying-off of Cultured Buttermilk.** LYNN R. GLAZIER AND H. G. LINDQUIST, Massachusetts State College, Amherst. *Milk Plant Monthly*, 30, No. 5: 27-30. 1941.

Of the three kinds of cultured milk sold in this country, acidophilus,

Bulgarian, and lactis, the latter is most popular. Wheying-off of finished produce during storage is one of its production problems. Factors affecting wheying-off are: purity of culture; fat content; salt balance; pasteurization temperature; ripening temperature; ripening acidity; method of breaking coagulum and the storage temperature.

It was found that the higher the developed acidity, the less was the curd separation and wheying-off during storage. At acidities of 0.68 per cent wheying-off occurred freely, it was less at 0.80, but at 0.87 to 0.93 per cent it occurred only after long periods of storage. Skim milk gave less desirable results than whole milk. From the standpoint of body and texture, a pasteurization temperature of 200° F. was found to be more desirable than 180° F. Lower temperatures of pasteurization should be avoided. Storage temperatures as high as 50° F. were unsatisfactory. More desirable results were secured at a storage temperature of 33° F. Wheying-off did not occur for some time. The turnover of cultured buttermilk should be as rapid as possible if wheying-off is to be a minor problem even under best production.

G.M.T.

**80. Six-day Delivery from the Plant Angle.** H. D. DRAIN, Peoples Dairy Co., Akron, Ohio. *Milk Plant Monthly*, 30, No. 6: 26-27. 1941.

See *JOURNAL OF DAIRY SCIENCE*, abstract No. 448, Vol. 24, Page A183, July 1941; also abstract No. 644, Vol. 24, Page A261, September 1941.

G.M.T.

**81. Homogenized Milk.** J. H. FRANSEN, Massachusetts State College, Amherst. *Milk Plant Monthly*, 30, No. 6: 24-27. 1941.

Advantages of homogenization stressed are: first, even distribution of the fat throughout the milk; second, marked improvement in the palatability of the milk; and third, increased digestibility of the milk. Emphasis is placed upon the necessity of a good quality milk for homogenization. Sales of homogenized milk are reported to be on the increase and will likely continue, provided milk selected for that purpose is of high quality, good flavor, and of low bacterial count and that no lowering of the fat content occurs.

G.M.T.

**82. Scientific Studies on Cooling Milk on the Farm.** J. ROBERTS, Investigator, Washington Committee on Relation of Electricity to Agriculture. *Milk Plant Monthly*, 30, No. 7: 36-40. 1941.

In a study of various methods of cooling milk, that by mechanical refrigeration was most satisfactory; cold air was practically worthless; water below 60° F. was satisfactory for meeting the temperature requirement of grade B milk; and proper use of ice maintained a milk of low bacteria count.

Agitation both of the milk and water in the mechanical wet storage method cooled milk from 94° F. to below 50° F. in 12 to 16 minutes. The ice requirement per 100 pounds of milk for four grade A producers during July and August was 107 pounds as contrasted to 41.6 pounds for grade B producers, the cost of cooling in the former case being 38 cents per hundred pounds of milk. Insulated tanks reduced the energy consumption by 50 per cent. G.M.T.

- 83. Cause and Prevention of Oxidized Flavor in Milk.** C. D. DAHLE, Penn. State College, State College, Pa. *Milk Plant Monthly*, 30, No. 9: 29-34. 1941.

Oxidized flavor, extremely prevalent in winter months and often attributed to pasteurization, is the most common and serious flavor defect in market milk today. The cause of this defect may be associated with individual cows, feeding practices, low bacterial count, metal contamination and sunlight. Prevention lies in rejection of offending milk, correct feeding practices, homogenization, high heat treatment, elimination of copper contamination and use of antioxidants. Data are presented as well as a brief review of the subject and research studies on the problem, listing 19 references. G.M.T.

- 84. What You Can Do with Colloidal Stabilizers.** W. C. COLE, Univ. California. *Food Indus.*, No. 9: 44-47. 1941.

Milk is a good example of a natural oil in water emulsion. The factors responsible for its stability are the same as in an artificial emulsion. The "membrane substance" that normally surrounds the fat globules in milk and thereby stabilizes the emulsion is different from any other substance in milk, but any one of several other milk constituents could likewise act as an emulsifying agent. Stable emulsions can readily be formed by homogenizing milk fat with skim milk or with solutions of any one of several proteins present in milk.

The author also discusses "Stabilizers of Foam."

J.C.M.

## PHYSIOLOGY

- 85. Action of Progesterone on the Genital Organs of the Unprimed Rhesus Monkey.** CARL G. HARTMAN AND HAROLD SPEERT, Dept. Embryology, Carnegie Instit. of Washington and Dept. of Obstetrics, Johns Hopkins Hospital. *Endocrinology*, 29: 639. 1941.

Development of the mammary gland with lobular proliferation of the acini was obtained following progesterone administration. The acini were greatly increased in number and the individual cells were larger and con-

tained increased amounts of cytoplasm and round vesicular nuclei. Numerous mitotic figures were seen. These changes were most marked after 32 days of treatment with 20 mg. of progesterone daily while similar but less striking growth effects were observed with 5 mg. daily for 32 days and 20 mg. daily for 27 days. R.P.R.

**86. Thyrotropic Hormone Content of Rabbit Pituitary During Growth.**

A. J. BERGMAN AND C. W. TURNER, Dept. Dairy Husbandry, Univ. Missouri. *Endocrinology*, 29: 313. 1941.

Results were presented concerning the amount of thyrotropic hormone in the pituitaries of male and female New Zealand White rabbits during growth. Animals were grouped at 500-gm. intervals and their pituitaries assayed in day-old White Leghorn male chicks. The average amount of thyrotropic hormone per pituitary and the concentration per gram of fresh pituitary tissue increased in groups of rabbits up to 2,500 gm. As the body weights increased above 2,500 gm. there was a decrease in pituitary thyrotropin content. The thyrotropin content of pituitaries from male and female rabbits was similar and this coincided with a similar growth rate in the male and female. R.P.R.

**87. Effect of Thyroidectomy of Young Male Goats upon Certain A.P.**

Hormones. E. P. REINEKE, A. J. BERGMAN AND C. W. TURNER, Dept. Dairy Husbandry, Univ. Missouri. *Endocrinology*, 29: 306. 1941.

Eight male kids were thyroidectomized between the ages of 5 and 24 days and killed after growth stasis had appeared (4 months). Pituitaries were removed and assayed for the lactogenic, thyrotropic, gonadotropic and sugar elevating factor. Similar assays were made on pituitaries from normal kids of the same weight (but younger) and from normal kids of the same age but of normal weight. Both the lactogenic and thyrotropic hormones were present in pituitaries of thyroidectomized kids in concentrations comparable to the normals of similar age. Gonadotropic hormone concentration was lowest in the pituitaries from thyroidectomized kids and the testes showed lack of stimulation. The sugar elevating principle concentration was lowest in pituitaries from thyroidectomized kids and their pancreas showed an abnormal histological picture. Liver weights were lowest in thyroidectomized kids. R.P.R.

**88. Assay of Posterior Pituitary Factors Which Contract the Lactating**

Mammary Gland. C. W. TURNER AND W. D. COOPER, Dept. Dairy Husbandry, Univ. Missouri. *Endocrinology*, 29: 320. 1941.

An assay method for the determination of the quantity of a factor present in the posterior lobe of the pituitary which causes the contraction of the

smooth muscles of the lactating mammary gland was described. A unit of the extract was defined as the minimal amount of substance which upon intravenous injection into an unnursed lactating rabbit would cause a minimal contraction of the mammary gland within 40 seconds. In commercial pituitrin a unit response was obtained with 0.001 U of the international standard of oxytocic hormone whereas in the pitocin reported to contain the same number of oxytocic units 0.00182 U were required. The assay of pitressin indicated a unit effect with 0.005 U of pressor hormone. It was suggested that either both oxytocic and vasopressin factors combine to cause the contraction of the mammary gland or a third factor is involved which is present in pitocin and pitressin in varying proportions. R.P.R.

- 89. The Effect of Estrogens, Gonadotropins and Growth Hormone on the Mammary Glands of Hypophysectomized Rats.** RALPH P. REECE AND SAMUEL L. LEONARD, Dept. Dairy Husbandry, New Jersey Agr. Expt. Sta., and Dept. Zoology, Rutgers Univ. *Endocrinology*, 29: 297. 1941.

Confirmatory evidence was presented which showed that injected estrogens would not induce mammary growth in hypophysectomized normal and castrated male, and castrated female rats. Endogenous estrogens produced by injecting pregnancy urine and menopause urine extracts or by hypophyseal follicle stimulating hormone likewise failed to stimulate the glands. In the latter group, the body weights increased slightly even though hypophysectomy was considered complete. Growth hormone alone was able to stimulate mammary growth to a slight extent in hypophysectomized males. Growth hormone injected simultaneously with estrogen resulted in mammary stimulation similar to that obtained with estrogen in normal males. In general there was a positive correlation between the increase in body weight and the degree of mammary stimulation when both hormones were injected. Sometimes the rats failed to grow but qualitatively the effects of estrogen were still manifested. It was suggested that either estrogen facilitates the action of the mammogenic factor contained in the growth hormone or that the growth hormone with its mammogenic factor facilitates the action of estrogen on the mammary glands. R.P.R.

- 90. Biological Assay of the Mammogenic Lobule—Alveolar Growth Factor of the Anterior Pituitary.** JOHN P. MIXNER AND CHARLES W. TURNER, Dept. Dairy Husbandry, Univ. Missouri. *Endocrinology*, 29: 324. 1941.

A technic was reported for the assay of the mammogenic lobule-alveolar growth factor of the anterior pituitary. A mouse unit of the lobule-alveolar growth factor was defined as the total amount of material required per mouse

when injected subcutaneously daily for 10 days to produce definite lobule-alveolar development in  $50 \pm 10$  per cent of 10 or more castrate, nulliparous, female mice weighing between 12 and 18 gm. Increasing dosages of various cattle pituitary lots did not show strictly quantitative relationships. When progesterone, however, was assayed very good quantitative results were obtained. R.P.R.

**91. Uterine Distention and Lactation.** JAMES T. BRADBURY, Bureau of Dairy Industry, U.S.D.A. *Endocrinology*, 29: 393. 1941.

Distention of the uterus with paraffin (melting point  $42^{\circ}$  C.) during the first 24 hours after normal delivery did not prevent lactation in the rat. It was believed that the operative technique was so hard on the mother that she neglected the new-born young until they did not have sufficient strength to suckle and died of starvation. When the postpartum rat was given a vigorous foster litter normal weight gains were observed. R.P.R.

**92. The Effect of Glycolysis Inhibitors and of Certain Substrates on the Metabolism and Motility of Human Spermatozoa.** JOHN MACLEOD, Dept. Anatomy, Cornell Univ. Med. College. *Endocrinology*, 29: 583. 1941.

Monoiodoacetate and fluoride inhibited the aerobic and anaerobic glycolysis of human spermatozoa and had a correspondingly depressing effect upon motility. Motility was found dependent upon the energy derived from the breakdown of sugar to lactic acid. No other substrates were effective. When spermatozoa were suspended in glucose-free Ringer's solution at  $38^{\circ}$  C. initial glycolysis was low and ceased completely within 3 hours. The normal level of glycolysis and rate of motility could be restored if a utilizable sugar was added to the medium within 2 hours after deprivation of substrate. Under anaerobic conditions lactic acid production in the presence of glucose remained linear for 4 hours but under aerobic conditions lactic acid production tended to decrease with time. R.P.R.

**93. Growth Response of Thyroidectomized Goats to Artificially Formed Thyroprotein.** E. P. REINEKE AND C. W. TURNER, Dept. Dairy Husbandry, Univ. Missouri. *Endocrinology*, 29: 667. 1941.

The effect of thyroidectomy on the growth and body conformation of young male goats together with evidence of alleviation of the symptoms by the oral administration of an artificial thyroprotein in small amounts was reported. Goats thyroidectomized during the first month of life reached a complete growth stasis one to two months after operation. Growth stasis was accompanied by typical symptoms of cretinism. The feeding of a thyroprotein, arrested the development of cretinism and stimulated nearly nor-

mal growth. Growth was roughly proportional to the amount of thyroprotein fed. R.P.R.

**94. Annual Variation in the Response of Crop-Sacs and Viscera of Pigeons to Prolactin.** ROBERT W. BATES AND OSCAR RIDDLE, Carnegie Instit. of Washington. *Endocrinology*, 29: 702. 1941.

The crop-sacs of 24 groups of 10 young White Carneau pigeons injected at intervals over a period of 2 years showed a semi-annual cyclic variation in their weight response to a 2.0 mg. (28 I.U.) dosage of the same prolactin preparation. The prolactin dosage used increased body weight over that of the controls by 4 per cent. Weight of pancreas was increased by 26 per cent; liver by 29 per cent; and intestine by 36 per cent. Testicular, thyroid, adrenal, and heart weight were not significantly affected by the treatment with prolactin while adrenal and heart weight showed no obvious cyclical or seasonal change. R.P.R.

**95. Failure to Find Sodium Pregnanediol Glucuronidate in Bull's Urine.** HERBERT S. STRICKLER, M. EVELYN WALTON AND DONALD A. WILSON, Dept. Chem., Univ. Pittsburgh and the Endocrine Lab., Elizabeth Steel Magee Hospital. *Soc. Expt. Biol. and Med. Proc.*, 48: 37. 1941.

No sodium pregnanediol glucuronidate was found in the urine obtained from the bladders of 4 freshly slaughtered bulls and 2 steers when Venning's method was used. The volumes of urine (1360, 840, 320, 300, 480, and 1060 cc.) were judged sufficient for demonstration of this substance if it were present in the amount suggested by previous reports for pregnanediol 3a, 20a from hydrolyzed bull's urine. R.P.R.

**96. Effect of Diethylstilbestrol Dipropionate on Mammary Development and Lactation.** SHEPPARD M. WALKER AND ALLAN J. STANLEY, Dept. Zoology, Louisiana State Univ. *Soc. Expt. Biol. and Med. Proc.*, 48: 50. 1941.

A castrate Jersey heifer at 12 months of age was injected over a period of 9 months with a total of 1560 mg. of diethylstilbestrol dipropionate and 530 mg. of testosterone propionate. At the end of 9 months injections were withdrawn and daily milking was begun. Milk secretion increased gradually for the first 50 days and then leveled off. The peak daily production during the first 100 days was 8 lbs. The administration of diethylstilbestrol dipropionate twice during the lactation period resulted in a sudden, slight increase in milk yield followed by a decrease and then an increase. Following the second treatment milk yield increased to 16 lbs. daily. A sterile 3-year-old heifer was injected with a total of 350 mg. of diethylstilbestrol



dipropionate over a 90-day period and milking then begun. Milk yield increased to 3.7 lbs. per day during the first 30 days. Diethylstilbestrol was then re-administered and milk yield eventually increased to 14 lbs. per day. The administration of diethylstilbestrol dipropionate to castrate and intact rats resulted finally in lactation suppression which was more pronounced in intact than in castrate animals. There was a premature opening of the vagina at 12 days of age of the young nursing treated rats which indicated that estrogen was secreted in the milk and also that the hormone was not readily attacked by any of the enzymes of the rat. R.P.R.

97. **Effects of Estrone and Progesterone on Male Rabbit Mammary Glands. I. Varying Doses of Progesterone.** WM. R. LYONS AND DANIEL A. MCGINTY, Div. Anatomy, Univ. California and Res. and Biol. Labs., Parke, Davis, and Co. Soc. Expt. Biol. and Med. Proc., 48: 83. 1941.

Groups of immature male rabbits were injected with varying doses (0.25, 1.0, 4.0, and 8.0 I.U.) of crystalline progesterone simultaneously with 120 I.U. of estrons for 18 injection days (injections made Monday through Friday). Of these 4 levels of progesterone, the 1.0 I.U. dose synergized best with the 120 I.U. of estrone, although lobule—alveolar development was not maximal. The 4.0 and 8.0 I.U. doses of progesterone appeared to inhibit mammary gland growth. R.P.R.

98. **Effects of Estrone and Progesterone on Male Rabbit Mammary Glands. II. Varying Doses of Estrone.** GEORGE SCHARF AND WM. R. LYONS, Div. Anatomy, Univ. California. Soc. Expt. Biol. and Med. Proc., 48: 86. 1941.

Six groups of immature male rabbits were injected 5 days weekly for 5 weeks with 30, 60, 120, 240, 480, and 960 I.U. of estrone respectively. The animals receiving 120 I.U. showed the best duct growth while the animals receiving 30 and 60 I.U. showed slightly less extensive duct systems and the higher levels caused cystic changes in the main ducts. The same 6 levels of estrone were injected into 6 other groups of male rabbits plus 1 I.U. of progesterone for a similar period. In every case lobule-alveolar growth of the mammary glands occurred and there was no tendency toward cyst formation even in the animals receiving the highest dosage of estrone. The best lobule-alveolar development was noted in the groups receiving 1 I.U. of progesterone plus 240 and 960 I.U. of estrone respectively. R.P.R.

99. **Dietary Requirements for Fertility and Lactation. XXVIII. The Lactation-Promoting Properties of Cystine when Added to Casein Diets.** B. SURE, Dept. Agr. Chem., Univ. Arkansas, Fayetteville. Jour. Nutr., 22: 491-498. 1941.

The lactation-promoting properties of lard, butterfat, hydrogenated

cottonseed oil, olive oil, and hydrogenated cottonseed oil in combination with wheat germ oil incorporated to the extent of 15 per cent on two types of salt mixtures were studied. The diets contained 17.7 per cent purified casein and 3.7 per cent protein derived from dehydrated bakers yeast as sources of the vitamin B complex.

Regardless of the nature of the oils or fats, or the composition of the salt mixture such diets did not meet the demands for lactation. When the diets were fortified with 0.2 per cent cystine lactation proceeded successfully and the young were weaned, although the diets supplied about 0.6 per cent methionine.  
C.F.H.

100. **Dietary Requirements for Fertility and Lactation. XXIX. The Existence of a New Dietary Factor Essential for Lactation.** B. SURE, Dept. Agr. Chem., Univ. Arkansas, Fayetteville. Jour. Nutr., 22: 499-514. 1941.

Diets adequate for normal growth of young rats may be deficient in factors required for reproduction and lactation. When diets supplemented by pure thiamine, riboflavin, pyridoxine, choline, pantothenic acid, nicotinic acid and "W" factor from liver extracts which did not support normal lactation were supplemented with Brewers' yeast, dried grass, liver extracts or rice bran extracts, efficient lactation resulted. When the rice bran extract was ashed no lactation activity was manifested which indicated that a new lactation factor was organic in nature. The author concluded that para-aminobenzoic acid or a related compound is a component of the new factor. Preliminary data suggest that inositol may also be a component.  
C.F.H.

101. **Implantation Following Mating in Hypophysectomized Rats Injected with Lactogenic Hormone.** EUGENE CUTULY, Dept. Anatomy, Wayne Univ. Soc. Expt. Biol. and Med. Proc., 48: 315. 1941.

Fourteen rats which had been hypophysectomized 1 to 5 days following mating were injected daily with 1 to 3 mg. of lactogenic hormone. Implantation failed to occur in 4 animals. Of the remaining 10 animals 2 carried to term or beyond while pregnancy was interrupted in 8 rats after 6-17 days. The results seemed to indicate that the lactogenic hormone was capable of stimulating corpus luteum function.  
R.P.R.

## MISCELLANEOUS

102. **Desiccation of Products Stored at Low Temperatures.** J. G. WOODROOF, Food Technologist, Georgia Agr. Expt. Sta. Refrig. Engin., 42, No. 6: 383. 1941.

Desiccation, or drying out, of stored frozen products is a major problem

of the frozen foods industry. Warm air coming in contact with refrigerating coils in a room gives up a part of its moisture, depositing it as frost on the coils. This less-than-saturated air coming in contact with the walls of the room and the surfaces of the product stored in the room tends to absorb moisture. On rising to the coils, the cycle is complete with additional moisture frozen to the coils. Thus the slowly moving air is the means of constantly drying the product.

Desiccation can be prevented only by protecting the product against surface evaporation. The rate of evaporation varies directly with the temperature and inversely with the relative humidity. A widely fluctuating temperature is the biggest single cause of drying out of frozen products. When this prevails there is a constant migration of water from the warmer portion of the container to the colder side. This may occur in a hermetically-sealed glass jar or can be evidenced by deposits of ice crystals on one side or under the lid. Methods of preventing desiccation in frozen products may be considered as:

1. Plant design—provide ante-room between any two rooms carrying temperatures more than 30° F. apart.
2. Provide humidifiers, as supplementary equipment.
3. Maintain as nearly as possible a constant and uniform temperature—never use storage room for freezing products.
4. Carry very low temperature—minus 20° F. or lower.
5. Use impervious materials as packages—as tin, glass, latex, coated cellophane, coated parchment, and others.
6. Surface treat or “glaze” the product during or immediately after freezing.
7. Avoid alternate thawing and refreezing the product in any degree whatever.
8. Avoid excessive circulation of air in the storage room. Strawberries and peaches are very susceptible to desiccation. The author found the most efficient treatment to be immersion freezing in invert syrup. In the case of strawberries, the sweet acid taste of the syrup blends with that of the berries. Glycerine has been found suitable for both fruits and vegetables in developing a glaze, it being practically inert to taste and can be used either as an acid or alkaline solution.

L.M.D.

103. **Air Motion in Refrigerated Spaces.** V. FLOYD SELF, Anemostat Corporation of America, New York, N. Y. *Refrig. Engin.*, 42, No. 5: 291. 1941.

The author indicates the necessity for creating a turbulence to break up incoming air mass into the multiplicity of smaller masses or streams and to bring other small masses—or streams—of the room air between the masses

of the incoming air. In refrigerating rooms the most practical method for obtaining intermingling or diffusion is to introduce the incoming cold air at the top of the room, enabling the force of gravitation to augment the static pressure which has replaced the energy of velocity, and to cause the conditioned air to flow down through the product, eliminating stratification. L.M.D.

- 104. Economic Operation of Batch Extractors.** W. L. FAITH, W. J. PETERSON AND MORTON SMUTZ, Kansas State College. Food Indus., No. 10: 43-45. 1941.

In recent years the addition of small amounts of chemical substances to foods has been increasingly important. Examples of this are the addition of vitamins and removal of alkaloids. J.C.M.

- 105. Four Basic Factors in Detergency.** FOSTER DEE-SUELL, Foster Dee-Suell, Inc. Food Indus., No. 10: 48-50. 1941.

Getting equipment and containers really clean is largely a matter of the correct choice and utilization of cleaners.

The known factors of detergency are: 1. Initial alkalinity or pH of the detergent solution. 2. Total alkalinity or buffer value of the detergent solution. 3. Effect in lowering of interfacial tension between the foreign matter and water. 4. Deflocculating and emulsifying power.

The author goes on to elaborate on these four points. J.C.M.

- 106. Why France Goes Hungry.** SIDNEY JAFFE, New York, N. Y. Food Indus., No. 9: 39-40. 1941.

Before the fighting began in the fall of 1939, farm labor was mobilized. These men lying idle ate heavily into the existing food supply. Then came the battle which destroyed electric systems throughout the country and smashed dairy farms.

One of the main reasons why Paris isn't getting milk is because there is not enough fodder to keep the cattle alive, and also because the cattle are slaughtered for want of feed and food for human use. J.C.M.

- 107. How To Tell What Color It Is.** GORDON W. McBRIDE, Washington, D. C. Food Indus., No. 9: 41-44. 1941.

The spectrophotometer, as long as it is in calibration, gives reproducible data and thereby provides a means of longtime comparison. This instrument can detect the slightest variation in color. J.C.M.

- 108. Lend Lease and Defense Increase Food Demand.** G. L. MONTGOMERY. Food Indus., No. 9: 62-63. 1941.

While increasing quantities of food are likely to be shipped to Great

Britain and other anti-axis countries, only certain products will be affected. Milk products will be in greatly increased demand, particularly butter, cheese and evaporated milk. Milk production in this country is the highest ever recorded. However, in spite of the favorable factors there is not going to be enough milk to supply the prospective demand. If milk is to be shipped abroad to meet the demand, consumption in this country will be restricted and dairy products processors will need to increase their manufacturing capacities. In spite of defense, it is likely that priorities will be granted for this purpose, considering the importance of dairy products among overseas food needs. J.C.M.

109. **Reliability of Organoleptic Tests.** J. W. CRIST AND H. L. SEATON, Michigan State College of Agr., East Lansing, Mich. Food Res., 6, No. 5: 529. September-October, 1941.

A mathematical consideration of the reliability of tasting and smelling which casts some doubt on the usefulness of "tasting panels." F.J.D.

# JOURNAL OF DAIRY SCIENCE

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Biochemical Journal	Journal of Veterinary Research
Biochemische Zeitschrift	Kaeseindustrie
Canadian Dairy and Ice Cream Journal	Kolloid-Zeitschrift
Canadian Public Health Journal	Lancet
Certified Milk	Le Lait
Cornell Veterinarian	Milchwirtschaftliche Forschungen
Dairy Industries	Milchwirtschaftliche Zeitung
Dairy World	Milk Dealer
Deutsche Milkerei Zeitung	Milk Industry
Endocrinology	Milk Plant Monthly
Food Industries	Molkerei Zeitung
Food Manufacture	National Butter and Cheese Journal
Food Research	New Zealand Journal of Science and Technology
Ice and Refrigeration	Oil and Soap
Ice Cream Field	Pacific Dairy Review
Ice Cream Review	Proceedings of Society of Animal Production
Ice Cream Trade Journal	Proceedings of Society of Experimental Biology and Medicine
Industrial and Engineering Chemistry	Refrigerating Engineering
Journal of Agricultural Research	Scientific Agriculture
Journal of Agricultural Science	Tierernahrung
Journal of American Medical Association	Tierzüchter
Journal of American Veterinary Medical Association	Zeitschrift für Infektionskrankheiten Parasitäre Krankheiten und Hygiene der Haustiere
Journal of Bacteriology	Zeitschrift für Physikalische Chemie, Abt. A and B
Journal of Biological Chemistry	Zeitschrift für Untersuchung der Lebensmittel
Journal of Dairy Research	Zeitschrift für Züchtung. Reihe B. Tierzüchtung und Zuchtungsbiologie
Journal of Dairy Science	Zentralblatt für Bacteriologie
Journal of Endocrinology	Züchtungskunde
Journal of Experimental Medicine	
Journal of General Physiology	
Journal of Genetics	
Journal of Heredity	

## SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland	Prussian Dairy Research Institute, Kiel, Germany
International Association of Ice Cream Manufacturers	State Agricultural Colleges and Experiment Stations
International Association of Milk Dealers	The Royal Technical College, Copenhagen, Denmark
National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

- 110. The Effect of Inhaled Substances on Milk Flavors.** W. E. PETERSEN  
AND J. G. BRERETON, Div. Dairy Husbandry, University of Minnesota.  
sota.

The effect of the inhalation of odors and vapors of 13 substances upon the flavor of milk was studied. The substances studied were placed or atomized into a specially constructed tent in which only the head and neck was entered. The effect upon milk flavors was ascertained by milk judges. The animals were subjected to the test substances for a period of two hours immediately before milking.

Inhalation of turpentine, paradichlorobenzene, camphor and vanillin caused flavoring of the milk characteristic of these compounds while inhalation of benzaldehyde, onion and garlic imparted a flavor to the milk that was not characteristic of these compounds. Inhalation of odors from corn silage, alfalfa silage and decomposing manure, caused "off flavors" in the milk, while synthetic orchid and scrapings from Roquefort cheese caused no detectable "off flavors."

- 111. Purification of Rennin from Commercial Rennin Extract: Properties of Purified Product.** C. L. HANKINSON AND L. S. PALMER, Division of Agricultural Biochemistry, Univ. Minnesota, St. Paul, Minnesota.

For abstract see JOURNAL OF DAIRY SCIENCE, 23, No. 6: 520. 1940.

- 112. Studies of Lipase Action. IV. The Inactivation of Milk Lipase by Heat.** VLADIMIR N. KRUKOVSKY AND B. L. HERRINGTON, Department of Dairy Industry, Cornell University.

Butter samples were churned from fresh cream pasteurized at 110°, 125°, 140°, 155°, 170°, and 180° F., with holding times ranging from 0 to 150 minutes. The rate of lipolysis during storage was measured by titration of free acids in the fat.

At 110° F., lipolysis was first activated and then reduced as the holding time was increased. The rate was reduced about  $\frac{2}{3}$  by holding 150 minutes.

At 125° F., the rate of lipolysis was reduced about  $\frac{1}{2}$  after 20 minutes (estimated) but it was still measurable after 150 minutes.

At 140° F., the rate of lipolysis was reduced more than half at zero holding time. The rate was measurable with a holding period of 15 minutes but not after 35 minutes.



At 155° F., the rate of lipolysis was scarcely measurable after zero minutes of holding.

**113. Studies of Lipase Action. V. The Effect of Storage Temperature Upon Lipolysis in Butter.** B. L. HERRINGTON AND VLADIMIR N. KRUKOVSKY, Cornell University, Ithaca, New York.

Samples of unsalted sweet cream butter were stored at a series of temperatures ranging from 32° F. to -15° F. for more than one year. The extent of lipolysis was measured at intervals by titration of the free acids in the fat. The data indicate that, in butter, lipolysis by the natural lipases of milk is inhibited at 5° F. or lower, though they are active at 30-32° F.

The data do not reveal any difference in the abilities of the formaldehyde tolerant and the formaldehyde sensitive enzymes to act at low temperatures.

**114. Studies of Lipase Action. VI. The Effect of Lipolysis Upon the Flavor Score of Milk.** VLADIMIR N. KRUKOVSKY AND B. L. HERRINGTON, Cornell University, Ithaca, New York.

A study was made of the relationship between the judges' scores and the "acid degrees" of raw milk within a few hours after milking.

The data indicate a threshold value for the recognition of rancidity at acid-degrees near 0.8. They also indicate that very slight degrees of lipolysis, such as is common in commercial market milk, may influence a judge's score without his being aware of the reason.

In the case of this particular pair of judges, and milk supply, statistical analysis shows that an increase of 0.33 acid degrees corresponded to a decrease of one point in the flavor score of the milk.

**115. Studies of Lipase Action. VII. The Influence of the Rate of Cooling Upon the Subsequent Rate of Lipolysis in Milk Stored at Low Temperatures.** B. L. HERRINGTON AND VLADIMIR N. KRUKOVSKY, Cornell University, Ithaca, New York.

The rate of lipolysis in milk stored at low temperatures depends upon the rate at which the milk was cooled before the storage period.

To secure a minimum rate of lipolysis, the cooling time should be reduced to a few seconds.

There is a critical temperature range in which the rate of cooling is most important. The upper limit of this range is approximately 20°-25° C. The lower limit is approximately zero in the case of natural milk, and approximately 10° C. in the case of temperature-activated milk.

**116. The Relation of the Use of Certain Antioxidants and Methods of Processing to the Keeping Quality of Powdered Whole Milk.** H. A. HOLLENDER AND P. H. TRACY, Department of Dairy Husbandry, University of Illinois, Urbana, Illinois.

All the antioxidants studied were found to retard the development of oxidized flavor in powdered whole milk but some were much more effective than others. The powders that developed the least amount of tallowiness under all conditions of treatment were those containing gum guaiac and hydroquinone. Powder made from milk preheated to 170° F. was more resistant to oxidation than that made from milk heated to either higher (190° F.) or lower (150° F.) temperatures. High moisture and high storage temperatures were conducive to oxidation.

Container features that favored retention of the normal flavor, color and solubility in milk powder were absence of air infiltration and the use of Avenex treated paper bags.

The sense of taste was more accurate than the peroxide test in detecting the early development of the oxidized flavor. The latter test was of no value in predicting the keeping quality of a fresh sample of powdered whole milk.

**117. Metabolism Stalls.** A. D. PRATT AND C. W. HOLDAWAY, Virginia Agricultural Experiment Station, Blacksburg, Va.

A description is given of four metabolism stalls which are built with sewer gratings in the rear of each and with funnels which direct the excreta into thirty-gallon garbage cans. Water cups, with a metered flow, and adjustable mangers are provided.

The room is heated for the convenience of the operator during digestion trials, by a hot water supply boiler which delivers the hot water to a Trane unit heater. A wall thermostat controls the fan in the Trane heater. An aquastat (reverse acting) in the return line between the Trane unit and the boiler actuates a water circulator.

Ventilation is accomplished by a Jamesway fan ventilator which starts when the temperature reaches 56° F. and stops at 52° F.

**118. The Bacteriology of Brick Cheese. II. Comparison of Washed-curd and Conventional Methods of Manufacture.** EDWIN M. FOSTER, JOHN C. GAREY, AND WILLIAM C. FRAZIER, Univ. Wisconsin, Madison.

In an effort to eliminate the common defect of excessive acidity with its accompanying effect on the body and flavor in high moisture Brick cheese a process was introduced for washing the curd with water before dipping. This procedure consisted of adding 25 to 28 pounds of water per 100 pounds of milk to the vat; then 50 to 55 pounds of whey were removed and replaced by an equal volume of water warmed to 90° F. The washing process removed about one-third of the lactose in the curd and fermentation of the remainder reduced the pH to the desirable range of 5.00–5.15 in cheese with a moisture content of 40–42 per cent. When the mixed starter of *Strepto-*

*coccus lactis* and *Streptococcus thermophilus* was used, all the lactose disappeared and the desired pH was reached in about one day.

The washing process had no noticeable effect upon the rate of development of the starter bacteria as detectable by cultural counts, but there was a slightly slower rate of acid formation in the washed-curd cheese.

The occurrence of undesirable fermentations was more pronounced in the washed-curd than in the conventional cheese, due probably to the relatively lower acidity in the former. However, the milk of good quality undesirable fermentations did not appear when the mixed starter was used; and the washed-curd cheese was superior to the other in flavor and body as well as in moisture content. The washed-curd cheese made from good quality milk had a mild and clean flavor, soft and smooth body and a medium close texture.

Late gas formation by anaerobic spore forming bacteria occurred in all cheese in which the pH did not drop below 5.3 during the first three days of ripening. This condition was most pronounced in cheese made with *Streptococcus thermophilus* starter by the washed-curd method from pasteurized milk.

**119. The Bacteriology of Brick Cheese. III. The Organisms Involved in Ripening.** EDWIN M. FOSTER, JOHN C. GAREY, AND WILLIAM C. FRAZIER, Univ. Wisconsin, Madison.

Brick cheese made from raw and pasteurized milk by the conventional and washed-curd methods with *Streptococcus lactis* and *Streptococcus thermophilus* starters alone and in combination was examined for the bacteria present during ripening. Cultural counts were made on samples taken at weekly intervals and representatives of the predominant organisms isolated at each sampling period.

Identification of 1040 cultures from eighteen lots of Brick cheese showed *Streptococcus lactis* predominant throughout ripening when it was used as the starter. It was also detected in cheese made with *Streptococcus thermophilus* alone, and with one or two exceptions eventually became predominant. *Streptococcus thermophilus* died out rapidly in cheese in which it was the starter and was seldom detectable in appreciable numbers after two or three weeks. Lactobacilli developed in all cheese made from raw milk, but usually were absent in pasteurized milk cheese. *Lactobacillus casei* was always the predominant rod form and usually was detectable after the second week of ripening. *Lactobacillus brevis* and *Lactobacillus lactis* were found in smaller numbers in several samples.

Streptococci other than the starter organisms frequently developed during ripening namely, *Streptococcus fecalis*, *Streptococcus bovis* and *Streptococcus liquefaciens*. The first two were particularly noticeable in cheese made from pasteurized milk. There was no regularity to the occurrence of

these organisms, hence their presence apparently depended upon the individual milk supply. When they did develop they usually were detectable after the first or second week of ripening.

**120. The Carotenoid Content of Milk Fat Fractions.** VLADIMIR N. KRUKOVSKY, Department of Dairy Industry, Cornell University, Ithaca, New York.

A study was made of the relationship between the carotenoid content and the physico-chemical properties of different milk fat fractions.

The data indicate an inverse relationship between the carotenoid content and the melting points of the fractions. They also indicate a definite relationship between the carotenoid content and the iodine numbers of the fractions.

The data suggest that the efficiency of absorption of carotene by an animal from its feed might be influenced by the degree of unsaturation of the fat present in the feed.

The flavor score of different fractions at the end of two years' storage at 4°-5° C. revealed that the intensity of the oxidized flavor varied inversely with the carotenoid content of the fractions. It appears that the substances responsible for the reduction in the susceptibility of the fat to oxidized flavor are concentrated in the liquid fraction.

The extreme low temperature fractions might well serve as the starting point in an attempt to identify the highly unsaturated acids and the anti-oxidant of butter fat.

**121. The Effect of High-Temperature Short-Time Forewarming of Milk upon the Heat Stability of Its Evaporated Product.** B. H. WEBB AND R. W. BELL, Div. Dairy Res. Lab., Bur. Dairy Indus. U. S. D. A.

High-temperature short-time forewarming fresh whole milk generally caused the heat stability of its 26 per cent solids evaporated product to be increased 2 times and occasionally as much as 6 times the stability of control samples. The control samples were forewarmed to 95° C. for 10 minutes. The test samples were forewarmed in a special tubular heater to temperatures between 101° C. (213.8° F.) and 165° C. (329° F.) with a heating time of 4 seconds, a holding time of 25 seconds, and a cooling time of 4 seconds. The relationship between the high forewarming temperature and the heat stability of evaporated milk differs with each milk. When a coming-up time of 4 seconds and a holding time of 25 seconds are used the optimum high forewarming temperature for most milk will probably fall between 120° C. (248° F.) and 140° C. (284° F.). Use of the optimum high forewarming temperature brought about, in the milks tested, a greater increase in heat stability in the evaporated milk than could be attained by

the addition of the optimum quantity of stabilizing salt to a normally fore-warmed milk.

- 122. Milk Lipase and Milk Flavor.** I. HLYNKA AND E. G. HOOD, Div. Chem. and the Div. Bact. and Dairy Res. Science Service, Dept. Agr., Ottawa, Canada.

The relation between lipase activity as indicated by surface tension measurement and flavor as judged by odor was studied. The results on a total of 144 milk samples including not more than 4 samples from one cow and representing 51 individual cows gave a correlation coefficient of .23. The role of milk lipase in average milk under ordinary methods of handling is discussed in relation to the flavor of milk and reference is made to raw milk cheddar cheese.

- 123. Increased Milk and Milk-Fat Production Following the Feeding of Artificially Formed Thyroprotein (Thyrolactin).** C. W. TURNER AND E. P. REINEKE, Univ. Missouri, Columbia, Mo.

Milk protein (thyrolactin) that had been iodinated to approximately the level at which optimal thyroidal activity is obtained was fed to lactating goats and cows and its effect on milk production was noted. When fed to goats at the rate of 5 to 10 grams daily for a 5 day period, thyrolactin stimulated increases in milk production ranging from 9.8 per cent and averaging 10.51 per cent. The heart rate was accelerated an average of 8.2 beats per minute. Fifty to 100 grams of thyrolactin fed daily to lactating cows for a 3 day period caused an average increase in milk yield of 8.59 per cent. Individual increases ranged from 6.09 to 22.6 per cent. In six trials in which milk fat analyses were made there was an average increase of 6.77 in fat percentage and 13.9 per cent in fat yield. Since the production of thyroidally active proteins is dependent upon proper control of the iodination process, it is recommended that iodoproteins to be used for the stimulation of lactation be produced by methods that have been proved to give satisfactory results, and further, that all such preparations be assayed biologically before being put to extensive use.

- 124. The Availability of The Iron of Cocoa and of Iron-Fortified Cocoa Mixtures.** FAYE KINDER, W. S. MUELLER AND HELEN S. MITCHELL, Massachusetts State College, Amherst.

The availability of the iron of cocoa and iron-fortified cocoa was determined by measuring the degree of hemoglobin regeneration in anemic rats when these substances were fed at the same level of iron intake as inorganic salt of iron. The effect of pure tannic acid was also determined. It was found that the iron of cocoa regenerated approximately two-thirds as much

hemoglobin as an equivalent amount of ferric chloride. Iron added to a cocoa mixture was found to be completely available, indicating that the factor which limited the availability of the iron of cocoa had no influence on added iron. Approximately two (1.7) per cent of pure tannic acid did not reduce the availability of the iron added to a milk ration. It is concluded that the fortification of cocoa or chocolate milk with iron may be warranted on the basis of the availability of the added iron and on the antioxidants which retard or prevent rancidity development in the presence of iron. However, the indiscriminate use of chocolate and cocoa in milk is not recommended because of the yet unexplained effect of cocoa on growth and intestinal function.

## BACTERIOLOGY

- 125. Plate Contamination in Milk Control.** K. P. LORENZ, Hanovia Chemical and Mfg. Co., Newark, N. J. *Milk Dealer*, 30, No. 12: 34-35, 79. September, 1941.

A discussion is given of how plate contamination can be prevented by means of ultraviolet air irradiation.  
C.J.B.

- 126. Variability in Streptococci of Group B.** J. M. SHERMAN, ELIZABETH CHASE GREISEN, AND C. F. NIVEN, JR., Cornell Univ., Ithaca, N. Y. *Jour. Infect. Dis.*, 69: 271-277. 1941.

Variations in cultures of group B streptococci have been found by observations over a period of several years on carefully studied stock cultures and by plating and re-isolating hundreds of daughter cultures for study. The characteristics studied were, ability to hemolyze blood and the ability to attack salicin and lactose. Although most of the strains proved to be remarkably stable, true variations were found in which daughter strains lost the characteristics studied and also variations in which daughter strains of negative parent strains acquired the characteristics with respect to salicin and lactose but not hemolysis of blood. The authors conclude that these characteristics are not sufficient in themselves for differentiation between species.  
J.F.C.

- 127. The Hemolytic Streptococci. Studies on the Carrier State in the San Francisco Area, with Notes on the Methods of Isolation and Serological Classification of these Organisms.** LOWELL A. RANTZ, Stanford Univ. School of Med., San Francisco. *Jour. Infect. Dis.*, 69: 248-253. Nov.-Dec., 1941.

Streptococci were isolated from 51.7 per cent of 345 excised tonsils and 30.2 per cent of 298 throat swabs taken a week before tonsillectomy. Group A streptococci were found in 32.7 per cent of the tonsils and 16.4 per cent

of the swabs; group B in 3.5 per cent and 1.7 per cent respectively; group C in 2.9 and 4.1 per cent; group F in 1.4 and 1.3 per cent; and group G in 3.8 and 2.0 per cent. Groups D, E, and H were not found in throat swabs and in only 0.3, 0.9, and 0.3 per cent, respectively, of the tonsils. J.F.C.

128. **The Selective Bacteriostatic Effect of Slow Oxidizing Agents.** W. L. MALLMANN, W. E. BOTWRIGHT, AND ELBERT S. CHURCHILL, Michigan Agr. Expt. Sta., East Lansing. *Jour. Infect. Dis.*, 69: 215-219. Nov.-Dec., 1941.

The slow oxidizing agents, potassium dichromate and sodium azide, were found to exert a greater bacteriostatic effect upon gram-negative bacteria than on gram-positive bacteria. By use of an appropriate concentration of these agents in culture media, the gram-negative organisms can be suppressed, thus facilitating the isolation of gram-positive organisms. The gram-positive cocci were found to tolerate the slow oxidizing agents in greater concentrations than were the gram-positive spore-bearing bacteria. Sodium azide was more stable than potassium dichromate, in that its effect was not destroyed by sterilization and its solutions deteriorated more slowly during storage. J.F.C.

129. **Bacteriological Air Analysis by the Cloud-Chamber Method.** S. D. ELLIOTT, London Hospital, London. *Lancet*, 241, No. 6166: 514-515. Nov. 1, 1941.

A method is described for isolation of the organisms from a sample of air in a fluid medium rather than a solid medium, as in the case of exposed plates and the air centrifuge. The essential features of the method are: First, the trapping of suspended particles by drawing the air sample through a water-vapor mist obtained by directing into a humidifying chamber a fine jet of steam, rapidly cooled by expansion to below 50° C.; second, the condensation of water on any particles left in suspension by cooling the saturated air; and third, by transferring known fractions of the washings from the chamber and the condenser into plates to be poured with a suitable medium. In experiments in which broth cultures of an easily identifiable streptococcus were sprayed into the room air to be tested, plugs of sterile cotton-wool, placed at the end of the system, for testing the sterility of the washed air remained sterile during all of the trials, indicating a high efficiency in trapping the organisms in the system. J.F.C.

## BUTTER

130. **Investigation on Diacetyl in Butter.** M. DE BUCCAR. *Ann. Hyg. Publ., Ind. Sociale*, 18 (N.S.), No. 7: 271-276. 1940.

Methods for the determination of diacetyl in butter are reviewed.

R.E.L.B.

## CHEESE

131. **Fermentation in Processed Cheese.** H. J. PALMER, Dairy Indus., 6, No. 9: 241. 1941.

In a study of the factors affecting fermentation in processed cheese it was found that minimum processing temperature of 165° F. is described as the "Critical Processing Temperature," below which the organisms which produce fermentations flourish in large numbers and above which few survive. The addition of 0.1–0.5 per cent potassium nitrate to processed cheese has a definite deterrent effect upon these organisms but the effect is much less than that of temperature. The use of the potassium nitrate along with a processing temperature of between 145–160° F. appears to be definitely helpful in retarding fermentation.

D.V.J.

## CHEMISTRY

132. **The Reduction Potential of Formaldehyde with Special Reference to the Microanalysis of Formaldehyde.** ROKURO AKANO AND MASAZO WATANABE, Inst. of Hygiene, Medical Academy of Kyoto. Mitt. med. Akad. Kioto, 30, No. 2: 629–643 (in German)–(in Japanese, 664). 1940.

Traces of HCHO can easily be detected in milk or in the milk distillate by means of the polarographic method using a dropping mercury cathode.

R.E.L.B.

## BY-PRODUCTS

133. **Plastics as Industrial Materials.** G. C. GRESS, Monsanto Chemical Co., Springfield, Mass. Refrig. Engin., 43, No. 1: 7. 1942.

A comprehensive review of the important types of plastic materials including thermosetting and thermoplastic kinds. Factors upon which plastics are evaluated to determine their suitability for any use which may be under consideration are: Mechanical properties, physical properties, durability and stability, moldability, and cost. Plastics have been used in the commercial refrigeration industry because they possess one or more of these characteristics: 1. Superior dielectric strength; 2. Superior mechanical strength; 3. Lower costs; 4. Chemical resistance; 5. Corrosion resistance; 6. Color or transparency; 7. Low heat transfer; 8. Decorative qualities.

Interior parts of domestic refrigerators are being made from polystyrene, a thermoplastic which does not become embrittled by low temperatures but, in fact, it increases in impact and tensile strength at 0° F. over that of 70° F. It also possesses high dimensional stability, and a low coefficient of expansion. Other properties commending it for refrigerator construction are: immunity to action of acids and alcohol, high heat insulation value, and its being odorless and tasteless.

L.M.D.



134. **Canned Whey Pudding—A New Product.** ANONYMOUS. Food Indus., 13, No. 11: 36-38. 1941.

Whey, the by-product from manufacture of cheese and casein, contains approximately half of the solids of milk, including nearly all the milk sugar, salts, albumin and water soluble vitamins. This paper describes the process of manufacture of another food product that may be added to a growing list of prepared foods containing whey solids. J.C.M.

## DISEASE

135. **Report and Comments Upon an Epidemic of Septic Sore Throat in McCook, Nebraska.** JAMES MEDFORD WILLIS, McCook, Nebr. Nebr. State Med. Jour., 26, No. 7: 248-251. 1941.

The epidemic of septic sore throat in McCook, with about 2000 cases and 11 deaths which occurred in the spring of 1934, was attributed chiefly to the milk from one dairy. In the case of several epidemics in this country the infected milk has been pasteurized by the flash method and the evidence in all has indicated that the milk was contaminated before pasteurization. Other epidemics of septic sore throat are also discussed. Apparently all investigators reporting epidemics of sore throat agree that the human type of *Streptococcus hemolyticus* is almost invariably the causative organism; the human carrier infects the cow's udder or a part of the udder producing mastitis and the milk from the infected cow infects the consumer. It is probable that many isolated or sporadic cases and small epidemics are never reported or suspected as being of milk-borne origin. Persons with sore throats or infections on the hands should never handle milk intended for human consumption. Efficient pasteurization is the best known preventive at the present time. R.E.L.B.

136. **A Practical Index of Mastitis.** J. G. DAVIS. Dairy Indus., 6, No. 9: 239. 1941.

A method is presented for detecting mastitis milk. It employs the use of catalase, rennet coagulation and solids-not-fat tests, the results of which are analyzed and a numerical index calculated. The calculation is based upon the following scale: 1 point is given for every centimeter of gas over 3 centimeters in the catalase test, 1 point for every  $\frac{1}{4}$  hour over  $\frac{1}{2}$  hour required for coagulation with rennet and 1 point for every 0.2 per cent S.N.F. below 8.8 per cent for Shorthorns (9.1 per cent for Guernseys and Jerseys, and 8.5 per cent for Holsteins). The sum of the points is interpreted as follows: 0-2 = negligible or nil; 3-4 = slight; 5-7 = definite; 8-10 = bad; over 10 = very bad.

The method has two main applications, namely, as a mastitis survey of

producers milk and as a means of correlating the extent of mastitis with the quality of the finished product after manufacture. D.V.J.

- 137. A City Health Officer Looks at Public Health.** JOHN L. RICE, Commissioner of Health, New York City. *Amer. Jour. Pub. Health*, 31: 1121-1127. 1941.

In a presidential address to the American Public Health Association, Dr. Rice states that health departments must assume leadership and responsibility for a broader field of public health.

In regard to milk control, Dr. Rice states, "Our programs of milk control need looking into. Probably too much effort is being placed on inspection of dairy farms and too little on the collection stations and on the pasteurizing plants. In many communities we have reached a point where the quality of the milk supply is such that the health department no longer needs to spend its time in setting and enforcing standards for different grades. Rather, the health department should spend its time in making sure that all milk offered for sale is safe." M.W.Y.

- 138. Milk-Borne Disease in Massachusetts 1933-1940.** ROY F. FEEMSTER, Mass. Dept. Pub. Health, Boston, Mass. *Amer. Jour. Pub. Health*, 31: 1169-1173. 1941.

Milk-borne disease continues to decrease in Massachusetts with only four outbreaks within the last five years. The milk-borne diseases occurring at the present time are largely limited to septic sore throat and undulant fever. Over 90 per cent of the milk consumed in the state is pasteurized. Progress in sanitary control is evidenced by the fact that 78 communities representing nearly 80 per cent of the population of the state, now have regulations requiring that all milk be either pasteurized or certified. M.W.Y.

- 139. Undulant Fever.** L. P. HIGHTOWER, Box 1719, Fort Worth, Texas. *Texas State Jour. Med.*, 36, No. 8: 542-546. 1940.

The public health problems now seem to be the eradication of Bang's disease from all dairy herds, the prevention of re-infection from all possible sources, and the further insistence on the sale and use of only pasteurized milk and other dairy products. R.E.L.B.

- 140. Etiology and Symptomatology of Brucellosis.** DEWITT NEIGHBORS, Med. Arts Building, Fort Worth, Texas. *Texas State Jour. Med.*, 37, No. 5: 353-355. 1941.

More than 10 per cent of the tested cows of the U. S. have shown positive agglutination tests for *Brucella* infections. The swine and goat varieties of *Brucella* may be disseminated through cow's milk. Human infection by

direct contact with animals is of frequent occurrence. Probably less than 5 per cent of individuals using diluted infected milk will develop clinical brucellosis. Consideration should be given to the contrasting symptoms and clinical course of acute and chronic brucellosis. R.E.L.B.

141. **Brucellosis, a Public Health Problem.** LUTHER L. TERRY, Dept. Pub. Health and Prev. Med. and Practice of Med., Med. Branch, Univ. Texas, Galveston. *Texas State Jour. Med.*, 37, No. 5: 359-363. 1941.

"Clean" or non-infected herds must be retested at frequent intervals in order to maintain their freedom from disease, and all contacts or additions to the herd must be known to be free of Bang's disease. The latest evidence supplied by the Livestock Sanitary Commission of Texas shows that 4.8 per cent of those cattle tested between 1934 and 1941 were positive reactors. However, there is an evident decrease in percentage of reactors where proper control measures have been instituted. Where a more careful evaluation of the incidence of swine infection has been made, a control program similar to that now being carried out with cattle may be found necessary. Pasteurization of all milk and milk products must be extended. When milk is used in rural areas from infected or potentially infected cows, there is a reason for advocating boiling of milk. The pasteurization of such milk products as butter and cheese should receive more serious consideration than it has in the past. The protection of slaughterhouse workers, veterinarians, dairy husbandrymen, etc., must be forwarded by education to the dangers of handling potentially infected animals, especially if there are abrasions on the hands or arms. The immediate disinfection of wounds inflicted while at work and the desirability of wearing rubber gloves when abrasions are present must be emphasized. Further development of vaccines now under study may offer greater hope than any measure now known to protect this group of individuals. R.E.L.B.

## FEEDS AND FEEDING

142. **The Effect of Feeding Some Fat Soluble Dyes to Milking Cows upon the Color of Milk Fat.** C. F. HUFFMAN AND C. W. DUNCAN, Michigan State College, Agr. Expt. Sta., East Lansing, Mich. *Quarterly Bul.*, 24, No. 1: 54-55. 1941.

It was found in this study that milk fat is readily stained by feeding certain soluble dyes. When Sudan III or IV were fed the color was noticeable in the next milking 12 hours later. When Brilliant Green was fed, the resulting butter was white with a green tinge. Upon melting, the butter oil was stained green. Perfect Purple also caused the butterfat to be green. Nigrosine Black produced a pink butterfat. It is apparent that certain

dyes are altered in the digestive tract or in the system of the cow. The possibility of using these fat-soluble dyes in studying the relation of food fat to milk fat is indicated.

P.H.T.

- 143. The Economic Utilization of Wheat.** A. J. AMOS. *Food Mfr.*, 16, No. 9. 1941.

Under normal peace time conditions England imports about 80 per cent of the wheat consumed in the country. This article discusses the advantages and disadvantages of producing white, "Wheatmeal," or wholemeal flour. The problem involves reduced materials for animal feeding.

J.C.M.

## FOOD VALUE OF DAIRY PRODUCTS

- 144. More Consumer Education—the Real Answer to More Milk in the Diet.** E. M. HARMON, National Dairy Council, Chicago, Ill. *Internatl. Assoc. Milk Dealers, Assoc. Bul.*, 34: 88-96. 1941.

Attention is called to a study by the National Dairy Council under the direction of Dr. C. W. Pierce to determine the effect of price upon milk consumption. Through various subsidies relief families in Boston were furnished milk through milk depots at five cents per quart. A similar basis was used in New York, Chicago and other cities. In each case the producer received a lower price, the dealer and retailer a lower margin and there was a subsidy by the Surplus Marketing Administration. The effectiveness in getting more milk consumed is answered by the fact that in Washington, D. C., only 25 per cent of those eligible to receive five cent milk actually went to the depots for it while in St. Louis only 12 per cent did so. It is argued that more consumer education is needed as much as cheaper milk. This contention is supported by findings of Gillett and Clark in a 1914 New York City survey of low income groups that a group not in contact with nutrition education spent 10 per cent of the food budget for milk and cheese while another group exposed to nutrition education spent 19 per cent. A similar comparison in 1928 gave 16 per cent and 25 per cent respectively for such groups. In one school with no nutrition program only 4 to 5 per cent of the students chose lunches which included milk and were rated adequate while in the other school with a nutrition program 30 per cent of the students chose adequate lunches. The avenues for education provided by the National Dairy Council and other agencies are outlined and it is suggested that their activities could with advantage be expanded.

E.F.G.

- 145. Milk Is in the Army Now.** JAMES P. JOHNSTON, Civilian Consultant to Quartermaster General, U. S. Army, Washington, D. C. *Internatl. Assoc. Milk Dealers, Assoc. Bul.*, 34: 9-13. 1941.

Everything possible is being done by the U. S. Army to increase the consumption of milk among our soldiers. The daily garrison ration per man contains eight ounces of fresh milk, one ounce of evaporated milk, two ounces of butter and a quarter ounce of cheese. In the army ration there is no oleomargarine. First prescribed in 1776 fresh milk then disappeared from the army ration entirely for 150 years. Now through daily experience many of our soldiers are learning what constitutes a balanced diet of which fresh milk is an important part. There is every reason to believe that new or at least improved food habits will be carried back to civilian life including that of drinking milk.

The first week at a milk bar at Ponchartrain Beach, New Orleans, milk outsold beer 2 to 1. This was followed by recommendations to the National Dairy Council that dealers be encouraged to establish milk bars at all thirty Army Recreation Centers. It is recommended that the best thought and most thorough cooperation be given to the Defense Industry Advisory Committees which will attempt to make effective the efforts of the dairy industry in the defense production program. E.F.G.

146. **Milk in Defense.** A. G. MARCUS, Internatl. Assoc. Milk Dealers. Internatl. Assoc. Milk Dealers, Assoc. Bul. 34: 3-8. 1941.

The problem is to increase consumption of dairy products in order to promote the nations health and at the same time to protect this effort from the well-intentioned though misdirected individuals who would substitute low cost foods as the price of milk rises. To meet the competition with some 300 other foods and beverages it is necessary to strive for lower costs of distribution to help get milk to consumers at a lower price. Particular attention is called to the work of the Public Relations committee through the Milk Industry Foundation in furnishing information, facts and statistics to various government defense agencies. It is suggested that members of the International Association of Milk Dealers as a matter of standard practice communicate with the Foundation in problems involving relations with any Federal agencies or departments.

The National Dairy Council is active in the nation wide nutrition program for defense. E.F.G.

147. **"Super-Iodized" Milk.** JOHN J. FOLEY, Washington Dairy, N. Tarrytown, N. Y. Milk Dealer, 30, No. 12: 39. September, 1941.

The Washington Dairy at N. Tarrytown, N. Y., found that what was at first thought to be an expensive undertaking was just the opposite. Iodized milk has increased sales and the community response has made them truly glad that they took the step.

By feeding organic iodine they have increased the iodine content of milk

from 420 parts of iodine to 1 billion parts of milk to 1,260 parts of iodine to 1 billion parts of milk.  
C.J.B.

- 148. Pasteurization and the Nutritive Value of Milk.** C. A. ELVEHJEM, Dept. Biochem., Univ. Wisconsin, Madison, Wis. *Milk Dealer*, 30, No. 12: 44-52. September, 1941.

For abstract see *Journal of Dairy Science* Vol. XXIV page A330 Nov., 1941.  
C.J.B.

- 149. Facts about Milk.** ANONYMOUS. *Milk Dealer*, 30, No. 12: 37-38, 84-85. September, 1941.

A collection of previously published or released statements concerning nutritional value of milk. The purpose of the article is to make readily available material that can be used to sell more milk.  
C.J.B.

- 150. Milk and Human Nutrition.** J. C. NISBET, Ohio Dairy Products Assoc., Columbus, Ohio. *Milk Dealer*, 31, No. 1: 154-158. October, 1941.

Milk and human nutrition are discussed under the following headings: Make-up of human body; maintaining the human body; milk and energy; milk for protein; milk for minerals; and milk for vitamins.  
C.J.B.

- 151. Refuel with Milk.** F. H. PLETCHER, Lab., Borden's Farm Products Co., Brooklyn, N. Y. *Milk Dealer*, 31, No. 1: 52-60. October, 1941.

The requirements of the body in terms of an optimum food consumption are summarized under four prerequisites as follows: 1. If the activities of life are to be normally performed an adequate amount of appropriate protein material must be furnished by the food. 2. If an optimum development of the bones and teeth is to be acquired in youth and the internal activities of the body are to be discharged a definite amount of ash or mineral constituents must be made regularly available. 3. A food supply must have present a sufficient quantity of the essential vitamins which have been found requisite for the normal functioning of tissues. 4. The greatest single demand of the body upon a food supply is that it furnish energy for the performance of the continuous, never-ceasing work which must be accomplished while life is present. The main carriers of energy for the performance of body activities are the fuel foods, the fats and carbohydrates and to a lesser extent protein material.

The author then relates how milk fulfills these prerequisites. C.J.B.

- 152. The Vitamin C Content of Dried Milk.** F. JUNG, Inst. Pharmacol., Univ. Berlin. *Klin. Wochschr.*, 19, No. 7: 153-155. 1940.

Fresh pasteurized bottled milk (3 samples), bulk milk (4 samples) and high-grade bottled milk (1 sample), which had a low bacterial count and was intended especially for infant feeding, contained 0.24–0.46, 0.32–0.98 and 0.43 mg. per cent ascorbic acid, respectively. These milks were purchased in the open market in Berlin. The vitamin C contents of various dried milk preparations, used in infant feeding, were determined: solutions of dried whole milk (14 g. milk powder in 100 g. water) contained 0.67–0.68 mg. per cent, solutions of dried buttermilk (10 g. milk powder in 100 g. water) 0.79 mg. per cent, and solutions of acid milk (14–16.7 g. milk powder in 100 g. water) 0.59–0.82 mg. per cent ascorbic acid. Transparent bottles, long transportation and prolonged refrigeration reduces the vitamin C content of fresh milk before it reaches the consumer. The vitamin C content of solutions of milk powder decreases on standing, although the vitamin C in the dried powder is quite stable in air. Irradiation with a mercury lamp, such as is used to increase the vitamin D content, reduces the content of ascorbic acid. The vitamin C content of dried milk powder is equivalent to that in Berlin whole milk. The vitamin C contents of both dried and fresh milk are too low to satisfy infant requirements and the use of ascorbic acid supplements is necessary.

R.E.L.B.

153. **Iodine Factor in Nutrition.** ORMSBY MCHARG, Iodine Products Co., New York, N. Y. Milk Dealer, 30, No. 11: 82–86. August, 1941.

The author points out the need for iodine in the daily diet and how it can best be acquired through iodized milk.

C.J.B.

### HERD MANAGEMENT

154. **Raising Herd Replacements.** E. K. BERG, Belle-Vernon Farms, Novelty, Ohio. Internatl. Assoc. Milk Dealers, Assoc. Bul., 34: 65–67. 1941.

Careful selection of both males and females on the basis of heredity is recommended and detailed advice is given on the care and feeding of the young. A case is reported where 150 different females purchased at auction privately averaged 2.9 lactations with only 15 per cent remaining in the herd at an average depreciation cost of \$28.75 per lactation while 100 home raised females completed 2.74 lactations with 55 per cent remaining and at a depreciation cost of \$16.50 per lactation.

E.F.G.

### ICE CREAM

155. **Cost of Pies.** PETER TRIMBORN, Adv. Mgr., Ice Cream Field, New York. Ice Cream Field, 38, No. 6: 6. 1941.

The typical pie making operations as carried out by Eliot Creamery Incorporated, Milton, Massachusetts, are shown by photographs.

It is stated that these pies wholesale for 28 cents each and dealers retail them for 39 cents each. The flavor of the centers is changed monthly, the most popular flavors being strawberry, butterscotch, cocoanut-pineapple, cherry, blueberry, and chocolate fudge.

Production costs are tabulated. These figures show a total cost of \$0.1673 excluding overhead. It is claimed ice cream pies stimulate winter sales and also enable the company to keep valuable help through the winter.

W.C.C.

**156. Developments in the Homogenization of Ice Cream Mixes.** J. C. HENING, New York Agr. Expt. Sta., Geneva, N. Y. Ice Cream Field, 38, No. 6: 14. 1941.

The importance of the homogenizer in the ice cream industry is pointed out and recent developments in homogenizers stressed. The results of several investigators are reviewed to emphasize the importance of many factors in determining the correct homogenization pressures to use as well as the conditions under which double stage and triple stage homogenization will likely be beneficial.

Mention is also made that rotary type machines do not give results equal to those obtained with the high pressure machines operating in the range 2000–3000 pounds pressure per square inch but they did compare favorably with those obtained in the range 1000–1500 pounds pressure per square inch. Results obtained by the author with a centrifugal colloidal mill gave coarse-textured ice cream.

W.C.C.

**157. How to Make Pies.** C. D. DAHLE, Pennsylvania State College, State College, Pa. Ice Cream Field, 38, No. 6: 7. 1941.

Ice cream pies now combine fruit and ice cream in a form so attractive, so palatable and at a price that the demand in certain markets is very great, it is claimed.

According to the author plants equipped for large scale production of pies generally follow this procedure. A paper plate is passed under a continuous freezer or hopper and the correct amount of soft ice cream dropped and formed as a crust on the plate. Next a filler drops a measured amount of fruit on the bottom "crust" after which ice cream from a continuous freezer or hopper is added for the top "crust." The pie is then decorated with an edging of whipped cream and a star or rosette may be placed in the center. It is then placed in an attractive package with a cellophane window.

Unless the plant is properly equipped the author questions the advisability of making these pies. In certain cases it is pointed out that the fruit centers are frozen in metal or paper containers of the correct diameter and are then cut to the desired thickness and placed in the soft ice cream "crust."

The proper relation of sugar content of filling to temperature of serving



must be maintained, and the stabilizer content (usually pectin) must be carefully regulated as a means of maintaining the desired hardness of the filling at the time of serving. Approximately 45 per cent sugar gave the desired results.

Satisfactory results were obtained with gelatin as a stabilizer for chocolate filling in pies it was stated. W.C.C.

**158. Ice Cream Pies.** KEN FORREST MCCRCH, Editor, Ice Cream Field, New York. Ice Cream Field, 38, No. 6: 32. 1941.

During the fall and winter months most ice cream is bought for home consumption, it is stated. That is the time to dress up your ice cream package, create new ideas and new combinations. Offer an ice cream that is delicious to eat, and in a form easy to serve besides having eye appeal is the recommendation of the author. It is stated further that many ice cream companies have found it profitable to make ice cream pies that retail generally at 33 cents to 39 cents each.

Pie-filling formulas and often advertising streamers may be secured from established supply houses. The author lists a number of such supply houses. W.C.C.

**159. Midwestern Dairy Trade Barriers.** C. A. IVERSON, Iowa State College. Internatl. Assoc. Ice Cream Mfrs., Proc. 41st Ann. Conv., 1: 85. 1941.

In general, trade barriers are said to fall into three different classes as follows: 1. Restrictions on trucks which are used in transporting ice cream or materials used for ice cream. 2. Licensing and registration restrictions on manufacture or sale of ice cream. 3. Trade barriers which restrict the areas from which dairy products going into ice cream may come.

The problems of trade barriers between states may be in part solved through the activities of the Council of State Governments. However, inspection regulations have the effect of trade barriers and are at fault because they lack uniformity as to sanitary requirements and as to the training and qualifications of inspectors. The U. S. Public Health Service Milk Ordinance and Code is of considerable value as a basis for standardization of inspection requirements. The International Association of Milk Sanitarians should also function in bringing about uniform and satisfactory standards for the inspection of dairy products. M.J.M.

**160. Barricades to Business.** W. H. LIST, JR. Internatl. Assoc. Ice Cream Mfrs., Proc. 41st Ann. Conv., 1: 76. 1941.

Trade barriers in the North Atlantic States are affecting the ice cream industry by retarding the free flow of dairy products in intrastate and inter-

state commerce, by creating artificial price structures for dairy products, by increasing inspection costs, and by causing dissatisfaction and confusion among producers whose milk and cream may be used in several markets. Trade barriers are raising the costs of dairy products for ice cream manufacturing. A comparison of the cost of cream in New York City in comparison to other leading markets of the North Atlantic States is attributed to the trade barrier effect of the New York City Board of Health regulation requiring cream for ice cream to come from locally inspected producers.

Constructive action is possible if approved creameries under one health jurisdiction should receive reciprocal approval from other consuming markets. Uniformity of regulations for inspection of all milk should be established, with producer inspection carried on by authorities in the producing rather than consuming areas. Any shipper of cream or butter should be allowed to sell his products in any market providing credentials of inspection and notice of intention to ship have been filed in advance. And finally, by enlightened public opinion much should be accomplished. M.J.M.

- 161. The Year's Work.** ROBERT C. HIBBEN, Washington, D. C. Internatl. Assoc. Ice Cream Mfrs., Proc. 41st Ann. Conv., 1: 35. 1941.

This comprehensive report of the activities of the International Association of Ice Cream Manufacturers, the Statistical and Accounting Bureau of the Association, and the Ice Cream Merchandising Institute, Inc., was published as a special bulletin of the I.A.I.C.M. entitled, "Outline of Activities for the Year 1941." An abstract of this bulletin recently appeared in the Journal of Dairy Science. M.J.M.

- 162. Address.** THE RIGHT HONORABLE MALCOLM MACDONALD, M.P., High Commissioner in Canada for the United Kingdom. Internatl. Assoc. Ice Cream Mfrs., Proc. 41st Ann. Conv., 1: 28. 1941.

The question of proper diet has become a crucial consideration for Great Britain in the present war. The British people are partly dependent upon food imports from other countries overseas, although every effort has been made to increase food production in England. Since the ships that are free to carry this food are limited, it is essential that the foodstuffs be concentrated and of high nutritive value. They should also come from as near at hand as possible, so that ships can make a maximum of trips. In this address the speaker stressed the importance of the program of the Dairy Industries of America in furnishing the necessary dairy products for the British people at this critical time. M.J.M.

- 163. Dairy Foods for Democracy.** L. E. HURTZ, Pres., Internatl. Assoc. Ice Cream Mfrs. Internatl. Assoc. Ice Cream Mfrs., Proc. 41st Ann. Conv., 1: 22. 1941.

The program of the dairy industry in producing dairy products for the United States and her allies is the subject of this discussion. The dairy products which have been shipped to Great Britain, and the future needs, are given. Since evaporated milk, cheese, and dry milk are the products suited to export shipment, the idea might be accepted that only a part of the dairy industry is affected. However, the structure of the dairy industries is so complex that any change in one branch affects the others. The ice cream industry can be especially helpful in the emergency by furnishing an outlet for part of the sweet cream remaining from the manufacture of dried skim milk. Lend-lease aid alone will increase the production of sweet cream, through dry milk manufacture, over 100,000,000 gallons. If no market for this excess cream existed, the farmer could not be paid a sufficient price for milk for drying to justify its production. Since milk will be produced in larger quantities than ever before, the processors and manufacturers must handle this supply efficiently and economically. M.J.M.

164. **Substitutes in Ice Cream.** L. J. HYNES. *Food Mfr.*, 16, No. 8: 84. 1941.

Since the war in England, the ice cream industry of Great Britain has been hard hit because ice cream is a milk solids product, and legislation has put a stop to their use in ice cream. This article goes into detail telling how the manufacturer can replace the solids by use of unsalted margarine and vegetable fats. Also the use of fillers such as starch from wheat flour and soya bean flour is discussed. J.C.M.

## MILK

165. **The Influence of Rancidity in Milk upon the Accuracy of the Fat Determination by the Mojonnier Method.** I. A. GOULD, Michigan State College, Agr. Expt. Sta., East Lansing, Mich. *Quarterly Bul.*, 24, No. 1: 19-22. 1941.

By aggravating lipolysis of the fat in milk through homogenization of the raw milk it was shown that the development of rancidity in milk would not only increase the fat acidity but also decrease slightly the percentage of fat as determined by the Mojonnier method. Increases in the fat acidity of 10-fold caused a reduction in the Mojonnier fat test of the milk of 0.0971 per cent. Larger errors in the Mojonnier results would be expected to occur in rancid products of high fat content. P.H.T.

166. **Influence of the Method of Sampling on the Accuracy of the Acidity Test of Sour Cream.** I. A. GOULD, Michigan State College, Agr. Expt. Sta., East Lansing, Mich. *Quarterly Bul.*, 24, No. 1: 42-49. 1941.

Analysis of 264 samples of sour cream for acidity were made on samples taken by three different methods. The methods used, together with the average results, are as follows: a. Weighed 9 gram sample—0.49 per cent; b. Use of 9 ml. pipette—0.423 per cent; c. Use of 9 ml. pipette rinsed with water—0.483 per cent.

The results indicate that for all practical purposes the use of a 9 ml. pipette will give sufficiently accurate results if the rinsings are included in the titration. When using this method the pipette should be rinsed with 7-9 ml. of clean, acid-free water. P.H.T.

**167. Analysis of Various Portions of Frozen Homogenized Milk.** G. M. TROUT, Michigan State College, Agr. Expt. Sta., East Lansing, Mich. Quarterly Bul., 24, No. 1: 31-36. 1941.

When creaming was inhibited by heating or by homogenization, the unfrozen portion was higher in fat and milk solids not fat than the frozen portion. In the case of unhomogenized milk the frozen portion was higher in fat but lower in solids not fat than the unfrozen portion. A pronounced settling of fat was noted in frozen homogenized milk. The wateryness at the surface was more pronounced when the milk was thawed slowly. Upon thawing homogenized milk exhibited no flakiness which commonly occurs in unhomogenized milk when frozen and thawed. When homogenized milk was frozen and thawed, a marked settling of the fat and solids not fat was noted, being greater when the thawing was done slowly. P.H.T.

**168. Short-Time High Temperature Pasteurization.** T. W. WORKMAN, Yale Univ., New Haven, Conn. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., No. 22: 585-588. Aug., 1941.

The various fundamental factors in a satisfactory pasteurization system are grouped under five headings. To determine how well the high temperature short time system met the requirements of satisfactory pasteurization twenty commercial plants of varied capacity operating with standard units of Cherry-Burrell, Creamery Package, Electro-pure and York were studied. Laboratory pasteurization at 160° F. for 15 seconds was effective in all cases using 17 strains of *Micobacterium tuberculosis*, 74 strains of *Brucella abortis*, *suis* and *melitensis*, 218 strains of Streptococci of human origin, and 186 strains of mastitis types of bacteria.

In the case of the plants studied temperatures and holding time were satisfactory, every particle of milk was properly treated and the resulting product was fully equal in creaming property, keeping quality and flavor to milk pasteurized by the standard holding method. Thermoduric types of bacteria may be destroyed in a lesser percentage, than with the standard holding method but these bacteria do not have public health significance and

such difficulty can usually be eliminated by a search for unsanitary conditions on some producers farms. It may be said that a short-time high temperature pasteurization: 1. Effectively destroys pathogenic bacteria; 2. Presents an entirely "closed" processing system; 3. Eliminates thermophilic development during processing; 4. Permits automatic control and the elimination of human element; 5. Yields final bacterial counts comparable with the holder process; 6. Presents some economic and operating advantages over standard holding equipment. E.F.G.

- 169. Short-Time, High-Temperature Pasteurization Using Tubular Heat Exchanger.** F. C. CLAUSON, Flynn Dairy Co., Des Moines, Iowa. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., No. 22: 581-584. Aug., 1941.

Experience in adapting a tubular heat exchanger with a direct expansion tubular cooler to the pasteurization of market milk is reported. With this method 1100 ft. of sanitary pipe and much other equipment was dispensed with and the new system occupied only about one third of the floor space of the old. High counts obtained at first were found to be due to thermodurics from improperly cleaned milking machines which were not killed by the new method of pasteurization. Elimination of this source of thermoduric bacteria resulted in milk of satisfactory bacterial count. To secure more uniform bacterial results two 600-gallon holding tanks were used so that milk from many producers could be mixed. Consumer reaction to the flavor of the milk produced by this method of pasteurization was very favorable. E.F.G.

- 170. Merchandising Value of Homogenized Milk.** G. G. DIFFENBACK, Abbotts Dairies, Inc., Philadelphia, Pa. Internatl. Assoc. Milk Dealers, Assoc. Bul., 34, 97-104. 1941.

The survey of the I.A.M.D. membership revealed 126 members in 111 cities now selling homogenized milk with seven others planning to enter the field. The highest average sales record was 60 per cent of the load by a concern selling at a one cent premium. Fifty-eight per cent of those reporting say that the major part of the homogenized milk supply is fortified with vitamin D and the usual premium for this milk is one cent.

The Philadelphia experience in introducing homogenized milk was very successful following a year of clinical study by the medical society on the nutritive properties of this milk in infant feeding. The favorable results of this study won the approval of the local physicians for the product. Homogenized milk is aided by the extensive promotion of evaporated milk on the basis of the desirable effects of homogenization. Fountain milk shakes are much improved when made with homogenized milk and increased consumption of milk shakes has in many cases been marked. E.F.G.

- 171. Repercussions of Defense Activities on the Milk Dealers Supply Problem.** JOHN L. WILSON, U. S. Agr. Marketing Service, Washington, D. C. *Internatl. Assoc. Milk Dealers, Assoc. Bul.*, 34: 55-64. 1941.

Due to the fact that approximately one per cent of our population is now in army camps and also because of the varying effects of export needs from various branches of the industry, there are now many shifts going on within the industry. For 1941 certain fluid milk production areas faced smaller supplies of roughage and higher grain costs. Other sections of the country were rather liberally supplied with both. In 1940 an equivalent of a half billion pounds of milk placed the United States on an export basis for the first time since 1922. A milk equivalent of 2½ billion pounds will be exported in 1941 with present plans for doubling that in 1942. Traditionally fluid milk plants have been the largest outlet for whole milk but indications point to manufacturing plants taking the lead in 1942. The price spread between fluid and manufacturing milk has narrowed. This is illustrated by the fact that by the latter part of 1941 Wisconsin farmers received 170 per cent of the 1930-1939 average for cheese milk, 160 per cent for condensery milk but market milk had advanced only to 116 per cent. It is expected that the development of new manufacturing milk areas will ease the pressure upon the fluid milk supply areas. Normally a dairy feed ration costs 2-2½ times as much in certain eastern fluid milk sections as in major manufacturing milk areas but this year the eastern producers are at an even greater disadvantage. Labor costs are also giving more trouble to eastern producers. Year to year changes in consumption of fluid milk are about half as great percentagewise as national income. Population shifts because of defense activity are also affecting the fluid milk demand. It is expected the milk dealer will continue to have serious supply problems for the duration.

E.F.G.

- 172. Some Competitive Aspects of Fresh and Evaporated Milk.** L. C. CUNNINGHAM, Cornell Univ., Ithaca, N. Y. *Internatl. Assoc. Milk Dealers, Assoc. Bul.*, 34: 68-79. 1941.

Over the last two decades the per capita consumption of fresh milk has been about constant, while that of evaporated milk has more than doubled. In low income areas particularly the consumption of evaporated milk has gained whenever the price spread between fresh and evaporated milk has widened. The 20-year average spread between New York Class I milk and Midwest condensery milk has averaged near \$1.00 per hundred to the producer. A study of comparative costs of production of Class I milk and condensery milk in two New York State areas indicate an added expense for Class I of about \$0.50 per hundred. The writer concludes that the historic spread and additional costs of producing fluid milk both are useful guides

in pricing fluid milk. The purchasing power of the consumer and the level of other foods must also be taken into consideration because too high a price for fluid milk will result in a shift to other alternatives. Over a period of years producers can expect only enough premium for fluid milk over evaporated milk to cover the cost of servicing the market. These added costs include mainly those due to meeting sanitary requirements, producing uniform year round supply and higher expenses of production in less favorable areas. E.F.G.

**173. How Some Ontario Dealers Solved the Special Delivery Problem.**

HAROLD P. HART, Oshawa Dairy, Ltd., Oshawa, Ontario. Internatl. Assoc. Milk Dealers, Assoc. Bul., 34: 39-44. 1941.

In Oshawa over a period of a month 72 per cent of 4000 milk customers did not use the special delivery service at all and 6 per cent of the customers accounted for an average of 74 of the 130 daily special deliveries. A one cent special delivery charge in 1934 reduced special deliveries by 30 per cent at the end of the first year and 34 per cent after the second year at an estimated annual saving to the Oshawa Dairies of \$5000 per year. Special deliveries began to increase again in 1940 but the Milk Control Board prohibited this service altogether on August 1, 1941. In Guelph a one cent charge per package was added beginning in 1936. Sales were not reduced and public opinion was favorable. London added a one cent charge February 1, 1941, cutting special delivery costs 75 per cent to 80 per cent. A "No Special Delivery" order is in effect now. Important facts are that a small per cent of the customers are responsible for most of the special deliveries and that a one cent charge is enough in depressed times but too low as conditions improve. E.F.G.

**174. Some Suggestions for Cleaning Equipment in the Plant.** GEORGE W.

COUGHLAN, Natl. Electric Mfg. Co., New York City. Milk Dealer, 30, No. 12: 86-87. September, 1941.

A brief discussion is presented on how to prevent corrosion in milk-plant equipment. Methods of preventing and removing milk stone are also given. C.J.B.

**175. Stepping up Milk Consumption in Industrial Plants.** W. LAWRENCE

WEAVER, E. I. DuPont de Nemours Co., Richmond, Va. Milk Dealer, 30, No. 12: 72-78. September, 1941.

A discussion is given of how milk consumption was increased in an industrial plant. This increased consumption has brought about a downward trend in illnesses and absences due to illness. The author concludes that all over the United States there are industries that need such a program.

The Dairy Council can show them the need and give them the materials with which to work.  
C.J.B.

- 176. Bacteriological Problems in Short Time High Temperature Pasteurization.** HAROLD WEINSS, York Ice Machinery Corp., York, Pa. Milk Dealer, 31, No. 1: 106-112. October, 1941.

Data are presented which show that the plate count and the methylene blue test do not indicate the quality of raw milk for pasteurization as they do not show thermoduric contamination. Further data are then presented to show that the resazurin test is a relatively accurate index of thermoduric contamination.  
C.J.B.

- 177. Some Principal Constituents of Normal Breast Milk.** M. WATANABE, K. KOBAYASHI AND Y. KATO, Inst. Hygiene and the Children's Clinic, Medical Academy of Kyoto. Mitt. med. Akad. Kyoto, 30, No. 4: 1077-1082 (in Japanese) (in German, 1345). 1940.

The milk of normal women in Kyoto contained 2.43 to 2.89 (average 2.64) g. total N, 0.46-6.33 (average 1.55) mg. Zn and 8.9 to 40.5 (average 22.0) mg. vitamin C per l. The specific gravity of the milk varied between 1.0287 and 1.0343 (average 1.0319).  
R.E.L.B.

- 178. Solids-Not-Fat Nomograph.** D. S. DAVIS, Wayne Univ., Detroit. Chem.-Anal., 30, No. 4: 80. 1941.

A nomograph is given for the estimation of the percentage of solids-not-fat in milk when the specific gravity of the milk and its percentage of milk fat are known. The nomograph is based on the equation of Hawley (Analyst, 58: 272. 1933):  $S.N.F. = 287.2(D-1)/D - 0.328 F$ , where  $S.N.F.$  denotes the percentage of solids-not-fat,  $D$  the specific gravity at 85°/60° F., and  $F$  the percentage of milk fat.  
R.E.L.B.

- 179. The Freezing Point of Carabao's Milk and Its Use in the Detection of Added Water.** M. GUTIERREZ, Nutrition Lab., Inst. of Hygiene, Univ. of the Philippines. Acta Med. Philippina, 2, No. 4: 497-510. 1941.

The average freezing-point depressions of 16 samples each of carabao milk and cow milk were  $-0.539^\circ \pm 0.00097^\circ$  and  $-0.542^\circ \pm 0.00218^\circ$  respectively; the difference between these figures is not statistically significant. The amount of water added to the milk can be calculated from the equations:  $y = -0.5318 + 0.0057x$  for carabao milk and  $y = -0.5375 + 0.0056x$  for cow milk, where  $x$  = per cent added water and  $y$  = the freezing-point depression. The straight lines described by these equations are given. A tolerance of 3 per cent added water is recommended. Higher values, found



cryoscopically, render it very probable that water has been added to the milk. The fats and solids-not-fat were shown to have no influence on the freezing-point depressions of carabao and cow milks and were also found to be less reliable as indices for the presence of added water. Acidity within the limits noted in this investigation (0.154–0.250 per cent for carabao milk and 0.138–0.210 per cent for cow milk) had little, if any, effect on the freezing-point depression.

R.E.L.B.

- 180. A Simple Method for Detecting Adulteration of Milk with Coconut "Milk."** M. GUTIERREZ AND P. SUNICO-SUACO, Food Lab., Inst. of Hygiene, Univ. of the Philippines. *Acta Med. Philippina*, 2, No. 3: 351–353. 1941.

The addition of coconut "milk," the milk emulsion obtained by expressing the grated meat of fresh coconuts, constitutes a unique and not uncommon method of adulteration of milk in the Philippines. The following method for detecting this form of adulteration is based upon the Selivanoff test for fructose, a constituent of coconut "milk" but not of animal milk. To a *fresh* solution of 0.1 g. colorless resoreinol (crystals of U.S.P. or reagent quality) in 10 cc. of 25 per cent hydrochloric acid are added 10 cc. of the suspected milk; this is mixed well and heated on the boiling water bath for 5 minutes. The development of a pink to red color indicates the presence of coconut "milk." Varying concentrations of coconut "milk," prepared by pressing through cheesecloth a mixture of 300 g. freshly grated coconut meat soaked in 500 cc. water for about 1 hour, were added to fresh carabao's or cow's milk. Milk which contained 6 per cent coconut "milk" gave a faint pink color with this test; this color became more distinct with concentrations of 10 per cent coconut "milk" or higher. With this test coconut "milk" alone gave a deep red color and precipitate, while carabao's or cow's milk gave a light brown color. Sucrose added to milk also gave a positive test but this form of adulteration can usually be detected by the sweet taste.

R.E.L.B.

- 181. Coliform Organisms and Pasteurization.** H. BARKWORTH. *Dairy Indus.*, 6, No. 5: 132. 1941.

The author reviews recent work with regard to the survival of coliform organisms during pasteurization. In summarizing the literature he concluded that even if certain strains of coli are capable of surviving pasteurization (145° F. for 30 minutes) it is clear from the evidence that for practical purposes survival can be ignored. Survival is obviously more a bacteriological phenomenon than a dairy problem and presence of coliform organisms in pasteurized milk is due to contamination after the heating procedure.

D.V.J.

**182. Sampling Producers Milk at Creameries.** S. B. THOMAS, R. H. WEEKS AND I. ROBERTS. *Dairy Indus.*, 6, No. 9: 235. 1941.

Sampling from the weigh-tank after all cans from a producer have been poured in, was found to be a satisfactory method. Checking each can separately is theoretically the best and is advocated for official sampling by Public Health Authorities. However, under commercial conditions this system is far too laborious and complicated.

No significant difference was found in the fat content, solids-not-fat content and resazurin reduction time of samples taken before and after mixing the milk in the weigh-tank. There was no detectable contamination from residual milk left in the tank after draining. D.V.J.

**183. The Bacteriological Control of Pasteurized Milk.** A. ROWLANDS AND A. L. PROVAN. *Dairy Indus.*, 6, No. 5: 134. 1941.

The authors recommend the phosphatase test for determining the proper pasteurization of milk. When unsatisfactory tests are obtained in bottled milk, checks should be made throughout the processing system to determine the source of the difficulty. As bacteriological tests on raw milk, the methylene blue or resazurin tests are suggested. The conventional plate count should be used on pasteurized bottled milk.

The modified Burri smear, which gives adequate information for commercial purposes, can be used in checking laboratory pasteurized samples.

Coliform tests are useful but in cases of slight contamination these organisms may pass undetected if tests are run immediately after pasteurization. This difficulty can be overcome by incubating the samples at 22° C. for 18-24 hours before testing.

The keeping quality test involves holding the pasteurized milk samples at 60° F. and tasting for development of taint and/or coagulation upon boiling. These observations are made at about one-half day intervals. A keeping quality of at least 3 days under those conditions is desirable. The development of a taint or coagulation in less than 2 days indicates a questionable bacteriological condition in the product. D.V.J.

**184. Food-Poisoning Epidemics.** ANONYMOUS. *Food Mfr.*, 16, No. 10. 1941.

A number of food-poisoning epidemics of the para-typhoid type have been reported in various parts of England. Synthetic cream has been suspected as being the cause.

Synthetic cream is a valuable war-time substitute for cream or artificial cream made from butter. It is unfortunately liable to act as a good medium for the growth of bacteria, therefore, the greatest care is necessary to prevent infection during preparation. Bacteria may be introduced in one or

all of the following ways: (a) By use of infected materials. (b) By improperly cleaned plant or containers. (c) By hands and clothing of workers. The article discusses how these ways of infection may be removed.  
J.C.M.

**185. The Dairying of the Future.** JOHN G. DAVIS. Food Mfr., 16, No. 11. 1941.

This article recommends certain sweeping alterations in the dairy industry, but it is emphasized that some of them may have to wait until the end of the war. The dairy industry is the most important aspect of the food supply and of agriculture. With a few exceptions the dairy industry is backward and suffers from a lack of up-to-date knowledge and co-ordination of its many aspects.

This article goes into the discussion of: Resting the Land; Inefficient Cows; Quality and Quantity of Milk; Inefficiency in Collection and Transport; and Problems of Small Retailer. The author makes several suggestions such as an Advisory Service; Payment on Quality Basis; and Dairy Education Centers.  
J.C.M.

**186. Preventing Oxidized Flavor in Milk.** D. V. JOSEPHSON AND C. D. DAHLE, Dairy Div., Pennsylvania State College. Milk Dealer, 30, No. 11: 29, 60-62. August, 1941.

A discussion is given of the use of cereal extract as a preventive of oxidized flavor in milk. The following conclusions are drawn: When 0.02 per cent of a concentrated extract of maize flour was added to milk which was subsequently contaminated with copper, a definite antioxygenic effect was noted. This retardation or prevention of the oxidized flavor was evident throughout the normal storage period for milk.

During seasons of the year when milk is somewhat susceptible to the development of the oxidized flavor, very small contaminations of copper from equipment are sufficient to produce the defect.  
C.J.B.

**187. Does a Co-operative Sales Program Pay?** HAROLD F. ALBERT, Triple Cities Milk Council, Binghamton, N. Y. Milk Dealer, 30, No. 11: 33, 64-66. August, 1941.

A discussion is given of the advantages of a cooperative sales program. From the results of actual experience in the triple cities the author believes that such advertising pays.  
C.J.B.

**188. Cultured Skim Milk or Buttermilk.** F. V. KOSIKOWSKY AND H. J. BRUECKNER, Dept. Dairy Industry, Cornell Univ., Ithaca, N. Y. Milk Dealer, 30, No. 11: 36-50, 71-74. August, 1941.

This is a report of a study of the factors influencing the quality of cultured skim milk and buttermilk. The following conclusions are drawn:

1. Viscosity of cultured skim milk was directly related to the acidity at the time of breaking the curd. As the acidity increased the viscosity of the cultured skim milk became greater.

2. Overripening of cultured skim milk, when incubated at 72° F., did not cause whey separation. On the contrary, as the acidity increased, whey disappeared in cultured skim milk that had been subjected to high pasteurization temperature.

3. Underripening, at the time the curd was broken, was directly related to whey separation until an acidity of 0.725 to 0.750 per cent had been reached.

4. Viscosity of the cultured skim milk was directly related to amount of M.S.N.F. in the skim milk. However, at an acidity of 0.70 per cent or lower no clear relationship existed.

5. Standardizing milk to different percentages of M.S.N.F. solids produced no significant results regarding whey separation in these studies.

6. A pasteurization temperature of 185° F. for 30 minutes produced the highest viscosity.

A pasteurization temperature of 150° F. for 30 minutes produced the lowest viscosity studied, while pasteurization temperatures of 205° F. and 170° F. produced a viscosity intermediate between 185° and 150° F.

7. A pasteurization temperature of 185° F. for 30 minutes produced the minimum amount of whey, while a pasteurization temperature of 150° F. for 30 minutes produced a maximum amount.

8. Holding periods of 60 minutes were not effective in changing the viscosity or improving the whey separation qualities of cultured skim milk when compared to holding periods of 30 minutes.

9. Pancreatic enzyme, when used in concentration of 1/15,000, was effective in reducing the viscosity of the cultured skim milk without increasing the possibility of whey separation.

10. Pancreatic enzyme, when added directly to whole milk prior to separation, was found more effective in reducing the viscosity than when pancreatic enzyme had been added directly to the skim milk. However, less whey was produced when pancreatic enzyme had been added to the skim milk.

11. The flavor score of the cultured skim milk was not lowered when pancreatic enzyme was used.

12. The amount of whey separated in the low range of acidity was directly related to length of storage period.

13. A storage temperature of 38° F. was more effective in preventing the separation of whey for a period of one to three days than a higher storage temperature. After this period of low storage temperature had no

apparent effect on the prevention of whey separation of the cultured skim milk. C.J.B.

189. **Milk Vending.** WILLIAM HESLIN, JR., Heslin Dairy Co., New Britain, Conn. *Milk Dealer*, 31, No. 1: 50, 130-131. October, 1941.

A brief discussion is presented on how the Heslin Dairy Company has profited from the use of milk-vending machines. C.J.B.

## PHYSIOLOGY

190. **The Treatment of Deficient Secretion of Milk by Irradiation with Ultrashort Waves.** N. HIRAMOTO. (The 42nd Convention of the Obst. and Gynec. Soc. in Japan, held in Osaka, March 26, 1941.) *Jap. Jour. Obst. Gynec.*, 24, No. 2: 37. 1941.

The breast and mid-brain were irradiated with ultrashort waves (6 m.). For irradiation of the breast a round pole, 12 cm. in diameter, was used. The distance of irradiation was 40 cm. Irradiation was applied from the back and front once daily, beginning with 5, 7, 9 and 10 minutes and then 10-minute irradiation was applied 4-10 times. For irradiating the mid-brain a round pole, 7 cm. in diameter, was used. The distance of irradiation was 35 cm. Irradiation was applied from the right and left sides of the head once daily for 5, 5 and 7 minutes, respectively, on 3 successive days. This treatment, when given to 15 puerperal women with deficient secretion of milk, decidedly accelerated the lactation. No ill effects appeared locally or in the entire body. This method became more effective when combined with other methods for promoting lactation. R.E.L.B.

191. **Effect of Visible Light on the Secretion of Milk.** KOTARO MENZU, Gynec. and Obst. Inst. Kitano Hospital, Osaka. *Jap. Jour. Obst. Gynec.*, 23, No. 3: 130-140. 1940.

Direct irradiation of the mammary glands of puerperal guinea pigs with red light promoted the secretion of milk, while irradiation with blue light resulted in retardation of the milk secretion; irradiation with white light had no effect. If the heads of puerperal guinea pigs were irradiated with red light 30 minutes daily for 8 days, the secretion of milk was accelerated, but similar irradiation with blue or white light produced no visible effect. Irradiation of the puerperal guinea pigs with red or blue light 1 hour after the subcutaneous injection of 1 cc. of 0.025 per cent methylene blue or 0.7 cc. of 1 per cent eosin per 100 g. body weight, respectively, gave no indication of any intensifying action. The effects of visible light on the secretion of milk are induced through the vegetative nervous system and the endocrine glands. R.E.L.B.

- 192. An Experimental Study on the Mechanism of the Secretion of Milk.**  
**I. An Experimental Study on the Physiological Growth of the Mammary Gland.** K. MENZU, Gynec. and Obst. Inst., Kitano Hospital, Osaka. Jap. Jour. Obst. Gynec., 22, No. 4: 257-264. 1939.

The number of secretory ducts in the mammary glands was small in infant rabbits but increased as maturity was reached. The ducts increased rapidly during pregnancy. The vessel walls usually consisted of a double layer of epithelium, but in the later stages of pregnancy and in the puerperium secretory ducts of medium or small size with a single layer of epithelium began to appear; fat was evident within the cells. The amount of interstitial tissue was in inverse proportion to the growth of the gland tissue. The "endoapparatus," "endochambers" and glandular lobes were usually absent in the mammary glands of the infant rabbits. A few "endoapparatus" but no "endochambers" were found in adult virgin rabbits. In the early stages of pregnancy the secretory ducts, "endoapparatus" and glandular lobes developed but not the "endochambers." In the later stages of pregnancy and the puerperium the "endochambers" showed quite rapid growth.

R.E.L.B.

- 193. An Experimental Study on the Mechanism of the Secretion of Milk.**  
**II. The Relation Between the Anterior Pituitary Lobe and the Mammary Gland.** K. MENZU, Gynec. and Obst. Inst., Kitano Hospital, Osaka. Jap. Jour. Obst. Gynec., 22, No. 6: 376-386. 1939.

Infant female rabbits and ovariectomized rabbits were given daily intravenous injections of 20, 50, 100, 200 and 400 R.U. (rat units) of gonadotropin or prachormon (total 280-840 R.U.). Daily injection of 20-200 R.U. of either hormone preparation resulted in a gradual increase in the growth of the mammary gland parallel to the dose injected; in the group which received 400 R.U. daily, however, the growth was about the same as in those which received 200 R.U. daily. The secretory development also increased gradually with the dosage up to 200 R.U.; it was extremely vigorous when 400 R.U. was administered daily. Gonadotropin and prachormon acted upon the mammary gland chiefly, though not wholly, through the ovary; the action of both preparations was identical. The growth of the mammary gland did not parallel the secretion when acted upon by the anterior pituitary hormones.

R.E.L.B.

- 194. An Experimental Study on the Mechanism of the Secretion of Milk.**  
**III. The Effect of the Separate and Combined Action of Follicular Hormone, Luteohormone and Anterior Pituitary Hormone On the Mammary Gland.** KOTARO MENZU, Gynec. and Obst.

Inst., Kitano Hospital, Osaka. Jap. Jour. Obst. Gynec., 23, No. 1: 25-34. 1940.

Daily subcutaneous injections of 100 I.U. of ovahormone benzoate (follicular hormone) for 21 days markedly accelerated the growth of the mammary glands in immature castrated rabbits; the growth was accelerated still further if 1 "K.E." oophormin (luteohormone) was also injected during the last 6 days. Daily injection of 1000 I.U. of ovahormone benzoate or of water-soluble ovahormone for 6 or 7 days into puerperal guinea pigs beginning on the first day of the puerperium inhibited lactation; ovahormone benzoate had a greater inhibitory action than water-soluble ovahormone. This inhibitory action apparently originates in the mid-brain. The subcutaneous injection of 1000 I.U. of ovahormone benzoate or of water-soluble ovahormone combined with the intravenous injection of 200 R.U. of gonadotropin daily for 21 days into immature, female, noncastrated rabbits produced a greater acceleration of the growth of the mammary gland than the administration of ovahormone benzoate or water-soluble ovahormone. If the rabbits which had received this treatment for 15 days followed by the administration of water-soluble ovahormone, gonadotropin and oophormin as above for 6 days were castrated 24 hours after the last injection, a marked acceleration of the secretory function appeared 72 hours later. Oophormin is particularly important for the growth of the "endoapparatus" of the mammary gland; it has practically no effect on lactation. R.E.L.B.

195. **An Experimental Study on the Mechanism of the Secretion of Milk. IV. The Relation between the Adrenal and the Mammary Glands.** KOTARO MENZU, Gynec. and Obst. Inst., Kitano Hospital Osaka. Jap. Jour. Obst. Gynec., 23, No. 1: 35-41. 1940.

Gradual atrophy of the mammary gland occurred in puerperal guinea pigs which had been separated from their young 2 to 4 hours after delivery; secretion was still active, however, even on the 6th day of the puerperium. Daily subcutaneous injections of 0.5 cc. of 0.1 per cent adrenaline hydrochloride inhibited lactation. Daily subcutaneous injections of 0.5 or 1.0 cc. interenin (cortical hormone) during the first 5 days of the puerperium inhibited lactation slightly. R.E.L.B.

### MISCELLANEOUS

196. **New Aids for Better Cleaning.** F. M. SCOLES AND MURIEL KEMP, Sheffield Farms Res. Lab., New York City. Internat'l. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., No. 22: 589-604. Aug., 1941.

It is pointed out that a formula for chemical cleaners may be satisfactory for some plants but not for others because of differences in the nature and extent of water hardness. In softening 3 waters of low, medium, and high

hardness it was found that for these samples sodium tetraphosphate was much more efficient than sodium hexametaphosphate or tetra sodium pyrophosphate. An explanation of the action of wetting agents or synthetic organic detergents is given and it is pointed out that synthetic organic detergents like soap have a polar or water soluble group on the end of a straight chain hydrocarbon while the wetting agents have the polar group nearer the middle of the chain.

Synthetic organic detergents which reduce the surface tension of their solutions so that suds are readily formed may be used to advantage in difficult cleaning operations. In can and bottle washers or where such solutions are pumped, excessive foaming might interfere. A prerinse plus slight soaking with the wetting solution, even sprinkling on with a brush, may be sufficient for many purposes. When laboratory tested, seven promising synthetic organic detergents gave cleaning results from very good to poor showing wide differences.

On the farm the producers cleaning problem can be greatly simplified by the use of the right synthetic organic detergent. For milk cans in the plant one of the polyphosphates may prevent calcium deposits on milk cans. Not to exceed 0.05 per cent of the right wetting agent may make a good can washing solution. Can washing with an acid solution has certain advantages over an alkali solution. For heaters treatment with acid solutions at 37.6° C. (100° F.) for half an hour followed by trisodium phosphate added to the same solution and circulated for 15 to 20 minutes may be used.

E.F.G.

197. **How to Develop Safe Drivers.** E. G. QUESNEL, The Borden Co., New York, N. Y. Internatl. Assoc. Milk Dealers, Assoc. Bul., 34: 83-87. 1941.

Safe driving is a combination of good physical condition, proper mental attitude, experience, training, knowledge, skill and action. Specific suggestions are given on selection of employees and their later training. The causes of accidents lie in the failure either of some one person or thing. Since the art of safe driving is little used by most people it must be developed and its practice encouraged. The 1942 winners of the vehicle accident prevention contest of the I.A.M.D. were announced at the end of this paper presented before the Sales and Advertising Section at Toronto, Ontario.

E.F.G.

198. **Helping Men Sell.** EARL W. BEEBE, II. P. Hood and Sons, Inc., Boston, Mass. Internatl. Assoc. Milk Dealer, Assoc. Bul. 34: 45-51. 1941.

Salesmen are best prepared by a thorough basic knowledge of their products. Then give them something different to talk about like: 1. Cello-



phane-wrapped butter, 2. Flower pots and ruby glasses, 3. Vacuum-packed cheese, 4. Butter dishes, 5. Cook books. These special packages effect permanent improvement in fluid sales. As a relaxation from delivery and collections it is suggested that one hour per day be used for competitive solicitation by route men. Unit money payment with a route building policy should be supplemented by periodic sales programs. E.F.G.

**199. Safe Practices for Dairy Salesmen and Drivers Accident Prevention Committee.** ANONYMOUS. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33: 3-12. 1941.

The subject is treated under two headings; first, safe driving practices and second, safe delivery practices. Under the former 34 situations likely to be hazardous to the dairy vehicle driver are discussed and suggested rules or procedures given. Under the second heading certain normally safe methods are given for handling bottles, cases, vehicles, cans and for certain delivery practices. The publication is intended as a manual for safety meetings of drivers and route men. E.F.G.

**200. Highlights of the New Tax Law.** J. S. SEIDMAN, C.P.A., Seidman and Seidman, New York City. Milk Dealer, 31, No. 1: 82. October, 1941.

The new tax law is discussed from the standpoint of tax on investment profit, excess profits tax, and new capital. C.J.B.

**201. Advances with Full-Time Health Leadership. Progress Reported in Middletown.** FRANKLIN M. FOOTE, Hartford, Conn. Conn. State Med. Jour., 5, No. 2: 116-121. 1941.

A brief discussion is included of food and milk supervision in Middletown, Connecticut. In 1939 the number of inspections of farms producing milk to be sold raw was more than doubled and the kind of inspection was improved. In 1937 approximately 40 per cent of the raw milk sold had a median bacterial count under 50,000 per cc.; in 1939 there was 84 per cent of such quality. The pasteurized milk sold increased from 51 per cent in 1937 to 61 per cent in 1939. A regulation requiring that caps on all grades of milk cover the pouring lip of the bottle went into effect on January 1, 1939. R.E.L.B.

**202. Refrigeration Requirements Affect Design of Water Cooling Equipment. Part I. Spray Ponds and Natural Draft Towers.** L. T. MART, The Marley Co., Kansas City, Kans. Refrig. Engin., 42, No. 6: 365. 1941. **Part II. Mechanical Draft Towers.** Refrig. Engin., 43, No. 1: 17. 1942.

A review of the trend in design of spray ponds, natural draft towers, and mechanical draft towers used in cooling water for refrigeration condensing systems and air conditioning systems. The mechanical draft tower has become increasingly important for water cooling, resulting in refinement in design of towers and increased capacity and efficiency in smaller units occupying less space. Diagrams and illustrations are included. L.M.D.

**203. FlakIce Machines in Units.** C. P. HOLLEY, York Ice Machinery Corp., Philadelphia, Pa. *Refrig. Engin.*, 43, No. 1: 23. 1942.

Two sizes of FlakIce machines are described, one DER-10, having capacity ranging up to 2000 lb. for 24 hours, and the larger DER-25, with capacity ranging from two to 5 tons. Freon-12 is the refrigerant employed in direct expansion in the freezing cylinder. The DER-10 machine is a package unit completely assembled at the factory, the only work necessary in the field being to make necessary electrical and water connections. The DER-25 machine, consisting of a freezing unit and separate condensing unit, must be assembled at point of field installation. The principal points of difference between these new models and the original FlakIce machine lies in their freezing cylinders being rigid in construction, not flexible. Ice is removed by an ice cutter made up of eight spiral blades whose action is of wedging effect rather than scraping in removing the ice layer which is 7/100 in. thick. Refrigeration is direct expansion in spiral grooves directly under the outer stainless steel shell of the freezing tube, the liquid refrigerant being fed through a shaft stuffing box, then through a drilled hole in the shaft to the spiral grooving and suction gas then is removed by means of similar connections at the other end of the cylinder. The ice leaving the freezing cylinders of these machines differs from that of the older machines in that its refrigerating effect is 144 b.t.u./lb. not having been subjected to any sub-cooling. L.M.D.

**204. The Role of the College Graduate in the Dairy Industry.** H. J. JUDKINS, President, A.D.S.A. *Internatl. Assoc. Ice Cream Mfrs.*, *Proc. 41st Ann. Conv.*, 1: 68. 1941.

The various opportunities for the college man in the dairy industry are presented. They are (a) Research and quality control, (b) Production supervision, (c) Plant construction and maintenance, and (d) Positions having to do with business management.

Thus far in the selection and training of college men vocational training has been overstressed. In order to avoid this it is suggested that college departments of Economics, Business Administration and Dairy Manufactures work out a combined program more suited to the needs of the industry.

In the selection of college men summer employment can be used as a trial

period. Where union labor contracts make it difficult to employ college graduates, the office, laboratory or sales department are points for starting these men. Once employed, they should be given an all-around training and should be watched. An employee experience record is suggested as a way of keeping in touch with the men. A suggested form for an employee experience record is given. M.J.M.

**205. Watch Your Water Supply—It Affects Food Quality.** K. G. WECKEL. *Food Indus.*, 14, No. 1. 1942.

Water can affect the quality of food products in a multitude of ways. The qualities that determine the worth of the water are: 1. Acceptable flavor and color; 2. Clarity, freedom from turbidity, and sediment; 3. "Inert" chemical properties; 4. Uniformity in composition; 5. Bacteriological acceptability; 6. Availability in volume; 7. Acceptable temperature.

This article cites examples where even water used for cleansing purposes is likely to leave deposits that change flavor, odor or color. Some of the things to guard against in water properties, and what to do to overcome water difficulties, are set forth in this article. J.C.M.

**206. Research Laboratory to Develop New Foods.** F. L. SEYMOUR-JONES. *Food Indus.*, 14, No. 1. 1942.

The Borden Company has recently installed a laboratory, complete with pilot plant equipment, on the 17th floor of its office building in New York. The purpose of the laboratory is to develop new food products, with choice of projects based on needs of sales department and capacities of production department. J.C.M.

**207. Control of pH in Canning Acid Foods.** C. T. TOWNSEND, AND M. J. POWERS. *Food Indus.*, 14, No. 1. 1942.

Sterilization of canned foods is a function of time and temperature. This applies to acid foods as well as to those requiring pressure cooks. It has been found that the pH concentration must be kept below 4.5 to prevent spoilage. Some products must have the pH carefully controlled. The author then discusses how the pH may be controlled. J.C.M.

**208. Bottled Gas.** ANONYMOUS. *Milk Dealer*, 31, No. 1: 42-43, 102. October, 1941.

A description is given of how a unique power installation, utilizing Butane gas, supplies refrigeration and heat at the Bluff View Dairy Company of Dallas, Texas. It is claimed that this gas method is saving from 10 to 35 per cent over former methods in a number of processes. C.J.B.

- 209. Licking Fleet Problems.** ANONYMOUS. *Milk Dealer*, 31, No. 1: 44-45, 118. October, 1941.

A discussion is given of how the Marin-Dell Dairy, wholesale dealer in San Francisco, has reduced the cost of operating its trucks by using correct size of tires, more careful battery service, and change in truck rear ends. Other savings are discussed under the following: Reducing deadweight, "check back" provided, road calls, oil dilution, and recording engine speed. Copies of the company's service record forms are shown. C.J.B.

- 210. British Food Industry Develops New Ideas.** ANONYMOUS. *Food Indus.*, 13, No. 11: 39-40. 1941.

Under the pressure of total war, England's food factories have worked out new products, processes, ingredients and equipment. For example, they are making more use of home meats such as rabbit, gray squirrel, venison and wild duck. J.C.M.

- 211. Birdseye Demonstrates New 20-Plate Froster.** ANONYMOUS. *Food Indus.*, 13, No. 11: 46-47. 1941.

This new 20-plate quick freezer represents an enlargement in capacity and improvement in control and efficiency of operation over the semi-commercial 10-plate unit described in *Food Industry*, September, 1940.

A freezing operation of this sort is adopted for large-volume operations in which the food is frozen in bulk prior to packaging. J.C.M.



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Certified Milk	Le Lait
Cornell Veterinarian	Milchwirtschaftliche Forschungen
Dairy Industries	Milchwirtschaftliche Zeitung
Dairy World	Milk Dealer
Deutsche Molkezeitung	Milk Industry
Endocrinology	Milk Plant Monthly
Food Industries	Molkerei Zeitung
Food Manufacture	National Butter and Cheese Journal
Food Research	New Zealand Journal of Science and Technology
Ice and Refrigeration	Oil and Soap
Ice Cream Field	Pacific Dairy Review
Ice Cream Review	Proceedings of Society of Experimental Biology and Medicine
Ice Cream Trade Journal	Refrigerating Engineering
Industrial and Engineering Chemistry	Scientific Agriculture
Journal of Agricultural Research	Southern Dairy Products Journal
Journal of Agricultural Science	Tierernahrung
Journal of American Medical Association	Tierzüchter
Journal of American Veterinary Medical Association	Zeitschrift für Infektionskrankheiten Parasitäre Krankheiten und Hygiene der Haustiere
Journal of Animal Science	Zeitschrift für Physikalische Chemie, Abt. A and B
Journal of Bacteriology	Zeitschrift für Untersuchung der Lebensmittel
Journal of Biological Chemistry	Zeitschrift für Züchtung, Reihe B. Tiersucht- und Zuchtungsbiologie
Journal of Dairy Research	Zentralblatt für Bacteriologie
Journal of Dairy Science	Züchtungskunde
Journal of Endocrinology	
Journal of Experimental Medicine	
Journal of General Physiology	
Journal of Genetics	
Journal of Heredity	

## SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland	Prussian Dairy Research Institute, Kiel, Germany
International Association of Ice Cream Manufacturers	State Agricultural Colleges and Experiment Stations
International Association of Milk Dealers	The Royal Technical College, Copenhagen, Denmark
National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

- 212. The Curd Number Test. A Method of Testing the Curdling Qualities of Milk.** BERNHARD SPUR AND IRVING J. WOLMAN, Milk Research Laboratory, Children's Hospital, Philadelphia, Pa.

The present paper records a fresh approach to the investigation of coagulation of milk in the human stomach and describes: 1. A device for reproducing more closely the actual happenings which take place within the human stomach following the taking of milk; 2. A standard procedure for producing, preserving and measuring the curds which form; and 3. An empirically derived scale for indicating the curd size distribution. The method utilizes the apparatus developed by Chambers and Wolman but with a routine of operation considerably altered from the initial report.

When a specimen of milk is made to coagulate within an artificial curdling device under rigidly controlled conditions, the curds which form manifest a size distribution which appears to be a constant physical characteristic of the milk undergoing test. After hardening, drying, sieving and weighing the masses of curds thus obtained and then applying to the weight data a so-called "a-b-c" formula empirically derived, it is possible to arrive at a "curd number" which epitomizes the milk's curdling qualities. This proposed technique for curd number has been subjected to critical analysis and found to be experimentally useful in problems dealing with the coagulating properties of cow's milk preparations as related to human digestion. Curd number has been found in general to run parallel to curd tension, but its approach is broader in scope and of greater applicability to research.

- 213. A Study of the Coliform Group in Ice Cream.** H. J. FOURNELLE AND H. MACY, University of Minnesota.

The coliform content of commercial ice cream and other frozen desserts was determined. The most probable numbers ranged from 0 to 9,180 per milliliter in factory-packed samples and from 0 to 101,000 per milliliter in scoop samples from retail stores. Brilliant green-bile broth proved to be a satisfactory presumptive medium. The following species were isolated: *Escherichia coli*, *E. coli* var. *acidilactici*, *E. coli* var. *neapolitana*, *E. coli* var. *communior*, *E. freundii*, *Aerobacter aerogenes*, and *A. cloacae*. The last-named species was most prevalent.

- 214. Curd Strength of Evaporated Milk.** J. C. MARQUARDT AND D. W. DENNISTON, New York (Geneva) Agr. Expt. Sta.

A study has been made with properly selected samples which defines re-



constituted evaporated milk in terms of its curd strength and creaming power.

Comparisons between the curd strength standards established by Hill and those of the American Medical Association and actual commercial homogenized milk and re-constituted evaporated milk have been made.

**215. A Statistical Study of the Influence of Moisture and Acidity on the Palatability and Fermentation Losses of Ensiled Hay Crops.**

T. E. WOODWARD AND J. B. SHEPHERD, U. S. Dept. Agr. Bureau of Dairy Industry.

Silage was made in different kinds of silos and from different kinds of grasses and legumes at the Beltsville Research Center. Comparisons were made of silages with high and low contents of moisture and with high and low acidities. In some of the silages the acidity was increased by molasses; in others, by hydrochloric and sulphuric acids. The effects of moisture and acidity were measured by the quality of silage as judged by the odor and by the quantity of dry matter cows would consume; they were also measured by the losses in the silo of dry matter, protein, and carotene. The odor of silage made from high moisture legumes was improved by all three treatments—a reduction of the moisture content, the addition of molasses, and the addition of acids. The quantity of dry matter that cows would consume was increased significantly by a reduction in the moisture content, and less significantly by the addition of molasses; while the addition of the acids greatly reduced the consumption of dry matter. The losses of carotene were significantly increased by a reduction in the moisture content.

**216. The Nutritive Value of Alfalfa Hay. I. Cystine as a Supplement to an all Alfalfa Hay Ration for Milk Production.** C. F. HUFFMAN AND C. W. DUNCAN, Mich. Agr. Expt. Sta., East Lansing.

Five lactating cows which had received alfalfa hay as their sole ration since calving and which had declined in milk production to the point where they were consuming larger amounts of total digestible nutrients than were required by a liberal standard were used to study the supplementary value of l-cystine on milk production. The addition of 20 gm. of cystine per day to the ration of each of four cows appeared to check the rapid decline in milk and fat production obtained on an all alfalfa hay ration but did not increase milk production significantly. The addition of 40 gm. of cystine per day to the ration of one cow resulted in a marked decrease in milk and fat production. Body weight increased but the consumption of hay decreased. The replacement of part of the alfalfa hay with isocaloric amounts of corn or barley resulted in significant increases in milk and fat production over the initial alfalfa feeding period and the subsequent cystine feeding period.

The results of this experiment indicate that cystine is not the first deficiency of an all alfalfa hay ration or of an alfalfa and corn starch ration.

**217. Seventy Years of Selection for Conformation in Dairy Cattle. A.**

A. LEWIS, Department of Dairy Husbandry, University of Missouri.

A study is reported of 5180 Commended and Highly Commended Island Jersey cattle and their parents. The data were tabulated in five period groups from 1866, including practically all calves registered during each period.

It was found that the proportion of the sires of Island Jersey cattle which were classified as Highly Commended increased from 64 per cent in 1866-82 to 82 per cent in 1890-3, and rose to the surprising proportion of 90 per cent in 1930-5. Only 29 per cent of the dams were HC in 1866-83, but over  $\frac{3}{4}$  were so classified in three of the following periods. There appeared to be a greater tendency to select HC bulls than heifers from HC sires and dams.

The mating of Commended sires to Commended dams declined from 27 per cent of the total matings in 1866-82 to 2.8 per cent in 1930-5. HC males  $\times$  C dams declined from 43.6 per cent to 17.5 per cent while HC  $\times$  HC matings increased from 21 per cent to 73 per cent in the same periods. There appeared to be assortive mating among the parents of registered bull calves in the 1866-82 and 1930-5 periods. The proportion of HC progeny to HC parents shows an increase, from 23 per cent to 43 per cent, between the first and second periods and thereafter considerable fluctuation but no consistent increase.

When the dams were only Commended about half as many calves were rated HC as when the dams were HC. Whether the sires were HC or C made little difference. Whether this was due to a greater genetic influence of the dam on her offspring or was a result of the selection practiced was undetermined.

**218. The Relationship of Errors in the Babcock Test to Losses in Cream Plants. J. L. HILEMAN, K. K. RUSH, AND CLARENCE MOSS, Dairy-men's League Co-operative Association, Inc., Syracuse, N. Y.**

The Babcock test gives a result that is too high in the case of both milk and cream. Because the error is proportionately greater in milk than in cream, a loss results. This loss increases with increasing fat content of the milk skimmed, and decreases with increasing fat content of the cream produced. It may vary from about 0.35 per cent where cream testing 50 per cent fat is made from milk testing 3.35 per cent fat, up to about 3 per cent where cream testing 20 per cent fat is made from milk testing 5 per cent fat.

The Babcock test for fat in skim milk shows only about one-seventh of the fat actually present according to the Mojonnier test. If this fat in the skim milk is ignored on butterfat accounting, it will be impossible to explain the loss of fat which it represents. This loss amounts to between 1.5 per cent and 2.0 per cent.

**219. Vitamin A and Carotene Requirements for the Maintenance of Adequate Blood Plasma Vitamin A in the Dairy Calf.** P. D. BOYER, P. H. PHILLIPS, N. S. LUNDQUIST, C. W. JENSEN AND I. W. RUPEL, University of Wisconsin, Madison.

Studies have been made to determine the blood plasma concentrations and the intakes of carotene and vitamin A necessary for the growing calf.

The data obtained showed that the blood plasma vitamin A was a more delicate measure of the state of vitamin A nutrition in the calf than either growth or blood carotene. A blood plasma vitamin A level of 10 $\gamma$  or more per 100 cc. was found to be necessary for adequate vitamin A nutrition of the growing calf. Blood plasma vitamin A levels of 7–8 $\gamma$  per 100 cc. were borderline levels while values below this were definitely inadequate.

Daily intakes of vitamin A which would maintain deficient, borderline, and adequate concentrations of blood plasma vitamin A were found to be approximately 6, 12 and 18 $\gamma$  per kg. of body weight respectively. The daily carotene requirements necessary to maintain an adequate plasma vitamin A and prevent deficiency symptoms were 75 $\gamma$  per kg. for Holstein yearlings and 125 $\gamma$  per kg. for Guernsey yearlings.

The blood plasma carotene levels which would maintain an adequate blood vitamin A were 50–70 $\gamma$  of carotene per 100 cc. for Holsteins and 110–140 $\gamma$  of carotene per 100 cc. for Guernseys.

**220. Comparative Palatability of Some Cereal Pastures.** A. O. SHAW AND F. W. ATKESON, Kansas State College, Manhattan.

To study the relative palatability of some cereal crops used for pasture, a five acre field was planted in four strips to Renò barley, Turkey wheat, common rye, and Balbo rye on October 19, 1939. Grazing observations were taken on six consecutive days, March 19–24, 1940, when the plants averaged four to six inches high. Two Holsteins, two Ayrshires and two Jerseys were used. Since the cows were fed grain, silage and hay at night, it was thought differences in palatability might be reflected more truly than if the cows were hungry. The production plane varied from two dry cows to one producing 70 pounds of fat monthly. The cows were started grazing on different strips each day. Length of time spent grazing by each cow on each strip was recorded at one minute intervals until all cows ceased grazing. The cows spent an average of 45 minutes or 52 per cent of the grazing time

on Balbo rye; 21 minutes or 24 per cent on common rye; 15 minutes or 18 per cent on wheat; and 5 minutes or 6 per cent on barley. A pronounced preference for Balbo rye and an evident dislike for barley were uniform for all cows. The average grazing time averaged about an hour and a half, and was quite uniform regardless of plane of production. The evident dislike for barley would be an important factor in considering its value in comparison with other cereals as a pasture crop.

## BOOK REVIEW

221. **Industrial Instruments for Measurement and Control.** THOMAS J. RHODES, The Procter & Gamble Co., McGraw-Hill Book Co., Inc., 1941.

This book, intended as a textbook for the formal study of the subject of instruments and automatic control in engineering schools, may well be used as a practical reference book for those concerned with instrument and control problems in industry.

The material contained in this book is broad in scope and comprehensive in treatment, and aside from one chapter (IV) on High-Temperature Pyrometry, it outlines the underlying theories upon which the operations of control instruments employed in the dairy and food industries are based, and quite thoroughly deals with operational applications. The chapter listings are as follows: Standards; Pressure and Vacuum Gauges; Indicating and Recording Thermometers; High-Temperature Pyrometry; Theory of Differential-Pressure Flow Meter Primary Measuring Instruments; Differential-Pressure Flow Meter Secondary Measuring, Recording, and Integrating Elements. Miscellaneous Inferential and Volumetric Flow Meters; Liquid-Level Measurement; Telemetering; Automatic-Control Theory; Automatic-Control Mechanisms; Miscellaneous Industrial Instruments. From this it can be seen that attention is given to methods of measurement and control under industrial requirements of four physical quantities, Temperature, Pressure, Flow, and Liquid Level. L.M.D.

## BACTERIOLOGY

222. **Variability in Streptococci of Group B.** J. M. SHERMAN, ELIZABETH C. CHASE AND C. F. NIVEN, JR., Cornell Univ., Ithaca, N. Y. Jour. Bact., 41, No. 1: 101. 1941. (Abs. of paper presented at annual meeting of S. A. B.)

Streptococci of Lancefield's group B are clearly defined physiologically and serologically, although diverse reactions occur in hemolytic power and ability to ferment lactose and salicin. The authors question whether strains are variants within the species, *Streptococcus mastitidis*, or should be given

separate recognition. The latter view is supported by the suggested specific names: *Str. mastitidis* (hemolysis +, lactose +, salicin +); *Str. agalactiae* (hemolysis -, lactose +, salicin +); *Str. opportunus* (hemolysis +, lactose -, salicin +); *Str. asalignus* (hemolysis +, lactose +, salicin -).

Pure cultures were plated and hundreds of daughter cultures were re-isolated in intermittently studying stock cultures. Hemolytic and non-hemolytic strains were obtained from the same culture. Salicin fermenting and non-fermenting daughter cultures were obtained from both originally fermenting and non-fermenting strains. Lactose-non-fermenting strains were isolated from bovine sources and several stock cultures lost their lactose-fermenting ability. Most cultures were stable with respect to lactose but positive and negative strains were obtained from three positive and one negative culture.

All variants were otherwise physiologically typical and were serologically identified as members of Group B. D.P.G.

**223. The Value of Certain Tests in the Differentiation of *Lactobacillus bulgaricus* from *Lactobacillus acidophilus*. J. M. SHERMAN AND H. M. HODGE, Cornell Univ., Ithaca, N. Y. Jour. Bact., 40, No. 1: 11. 1940.**

Here are presented differential tests applied for more than twenty years. Recent experiments confirm the validity of earlier methods and these tests correlate with differential methods applied by others.

The authors summarize the differentiation thus: "*Lactobacillus bulgaricus* is unable to make repeated growth in a lactose-peptone-yeast extract broth, is unable to grow in media containing 2.5 per cent sodium chloride, and cannot grow in broth with a reaction of pH 7.8. *Lactobacillus acidophilus*, on the other hand, is not inhibited in such mildly alkaline or saline media, and grows well through ten successive transfers in the relatively simple broth.

"*Lactobacillus bulgaricus* rarely grows at 15° C. whereas *Lactobacillus acidophilus* usually grows at this temperature. At 50° C., *Lactobacillus acidophilus* apparently never grows; newly isolated strains of *Lactobacillus bulgaricus* nearly always grow, though old laboratory cultures frequently fail."

Emphasis is given to the statement that no claims are made for the tests if applied to other closely related species. D.P.G.

**224. Physiological Characteristics of Lactic Acid Bacteria Near the Maximum Growth Temperature. I. Growth and Acid Production. ROBERT M. STERN AND W. C. FRAZIER, Univ. of Wisconsin. Jour. Bact., 42, No. 4: 497. 1941.**

In some commercial processes, as in the manufacture of Swiss cheese,

bacteria must not only survive high temperatures but must grow at near-maximum temperatures. For *Lactobacillus bulgaricus*, 37° C. and 49.5° C. were chosen as optimum and near-maximum temperatures. Growth was observed by turbidity measured by an Evelyn photoelectric colorimeter with a 7,200 Å filter.

With *L. bulgaricus* at 37° C. there was close relationship between the rate of lactic acid production and the rate of growth, but at 49.5° C., as much acid was produced after reproduction had ceased as was produced during growth.

Differences resulting from variations in the size of inoculum of *L. bulgaricus* were apparent only during the early stages of growth at 37° C. An increase in the number of organisms present in the inoculum tended to shorten the lag phase, but had no apparent effect on the maximum amount of growth obtained. At 49.5° C., when larger inocula were employed, the increase in growth was more rapid than when smaller inocula were used, and the maximum population obtained depended on the number of cells added at the start of the experiment.

With *L. bulgaricus* inocula of various ages there was an increase in the length of the lag phase coincident with an increase in the age of the inoculum but the total amount of acid produced at the end of 24 hours was the same, although less growth was observed in the medium inoculated with older cultures.

When *Streptococcus thermophilus* and *L. bulgaricus* were grown at near-maximum temperatures, the production of volatile acids was affected only slightly. *L. bulgaricus* produced more volatile acid at 49.5° C. than at 37° C. When these two organisms were grown at near-maximum temperatures, there was no difference in the distribution of the two isomers of lactic acid from that found at optimum temperatures. D.P.G.

## 225. Physiological Characteristics of Lactic Acid Bacteria Near the Maximum Growth Temperature. II. Studies on Respiration.

ROBERT M. STERN AND W. C. FRAZIER, Univ. of Wisconsin. Jour. Bact., 42, No. 4: 501. 1941.

The rate of respiration is stimulated and respiratory enzymes are soon inactivated at temperatures near the maximum for growth. Differences were found in the rate of oxygen uptake of resting cells of two strains of *Streptococcus thermophilus*.

The growth curve and the oxygen uptake curve of *Lactobacillus bulgaricus* were similar during the period of active growth at 37° C., but respiration continued at a rapid rate after reproduction had ceased. At 49.5° C. the rate of oxygen uptake was rapid during the early stages of growth, but after several hours there was a marked inactivation of the respiratory mechanisms, followed by a decrease in the growth rate. D.P.G.

**226. The Lactic Acid Fermentation of Various Kinds of Streptococci.**

PAUL A. SMITH AND J. M. SHERMAN, Cornell Univ., Ithaca, N. Y.  
Jour. Bact., 41, No. 1: 101. 1941. (Abstract of paper presented at annual meeting of S. A. B.)

In a study of the production of lactic acid from glucose, 147 cultures representing the better-known groups and varieties of streptococci were used. The average percentages of lactic acid produced from glucose by four groups of the streptococci were: pyogenic streptococci, 82.1 to 88.4; viridans streptococci, 89.9 to 93.6; enterococci, 91.2 to 96.0; lactic streptococci, 93.7 and 96.6.

"The slightly lower average efficiency of the pyogenic streptococci is of possible significance, although the overlapping of individual cultures makes this conclusion doubtful."

D.P.G.

**227. Stimulation of the Growth of Lactobacilli by Extracts of Streptococcus lactis and Streptococcus cremoris.** P. ARNE HANSEN. Biotech. Chem. Lab., Copenhagen, Denmark. Jour. Bact., 41, No. 1: 41. 1941. (Abs. of paper presented at annual meeting of S. A. B.)

Extracts of autolyzed cells of several strains of *Streptococcus lactis* and *Str. cremoris* were prepared. When added to milk these extracts raised the endpoint of fermentation for *Lactobacillus casei*. "This effect may be of significance in explaining the predominance of this type of lactobacilli in cheese, a medium in which lactic streptococci develop abundantly at first and then die and disintegrate."

D.P.G.

**228. The Activity of Vitamin B<sub>6</sub> Analogues for Lactic Acid Bacteria.**

NESTOR BOHONOS, BRIAN L. HUTCHINGS AND W. H. PETERSON. Univ. of Wis., Madison. Jour. Bact., 41, No. 1: 40. 1941. (Abs. of paper presented at annual meeting of S. A. B.)

Using a synthetic basal medium it was possible to determine the vitamin B<sub>6</sub> requirements of various species of lactic acid bacteria as well as the biological activity of various analogues of vitamin B<sub>6</sub>.

Vitamin B<sub>6</sub> was found to be essential for growth and acid production by some lactobacilli, stimulatory for one and non-essential for others. It was shown that three species of *Lactobacilli* synthesize vitamin B<sub>6</sub>.

D.P.G.

**229. The Production of Bactericidal Substances by Aerobic Sporulating Bacilli.** RENE J. DUBOS AND ROLLIN D. HOTCHKISS, Hospital of the Rockefeller Inst. for Med. Res. Jour. Expt. Med., 73, No. 5: 629. 1941.

In this paper further reports are given on the nature and action of the bactericidal substances produced by *B. brevis*, *B. subtilis* and other aerobic

sporulating bacilli. Cultures isolated from soil, manure, sewage and cheese were found to be endowed with properties antagonistic to *Staphylococcus aureus* and *Escherichia coli*. Material suspected of containing such antagonistic bacteria was heated to 70° C. for 30 minutes to destroy the non-sporulating forms present. This heated material was then inoculated into suspensions of living cells of *E. coli* and *S. aureus*. The activity of the antagonistic flora was determined by frequent microscopical and cultural tests.

The antagonistic cultures of aerobic sporulating bacilli all yielded an alcohol-soluble, water-insoluble fraction endowed with bactericidal activity. The name tyrothricin has been proposed for this fraction. Tyrothricin has yielded two crystalline products. One of these substances has been called gramicidin because of its selective bacteriostatic and bactericidal effect against gram-positive microorganisms. Gram-negative bacteria are resistant to gramicidin. Intraperitoneal injection of gramicidin exerts a protective action against infection of mice with pneumococci and streptococci. Gramicidin, when applied locally at the site of the infection, retains *in vivo* a striking activity against gram-positive microorganisms. Little or no hemolytic effect was observed with gramicidin. The other crystalline product yielded from tyrothricin has been called tyrocidine, so named partly because the substance is rich in tyrosine. Tyrocidine, when tested in buffer solution in the absence of broth, affects both gram-positive and gram-negative species. Tyrocidine causes immediate hemolysis of washed red cells of the rabbit. Tyrocidine is essentially ineffective *in vivo*. Crystalline preparations can exert a definite protective action against pneumococcus infections in mice, but all attempts to obtain a protective effect with tyrocidine against gram-negative infections have so far failed. C.N.S.

**230. The Probable Identity of Diphtheroids Isolated from Aseptically-drawn Milk with *Corynebacterium bovis* and *Bacterium Lipolyticum*.** L. A. BLACK, Univ. of Maryland, College Park, Maryland. Jour. Bact., 41, No. 1: 99. 1941. (Abs. of paper presented at annual meeting of S. A. B.)

Seventy-one cultures of diphtheroid bacilli were isolated from milk and other animal sources, including 7 different species of *Corynebacterium*. All cultures from milk were alike and appeared to be identical to *Corynebacterium bovis* (Manual of Determinative Bacteriology, Fifth Edition). Representative cultures of the diphtheroids from milk caused rancidity in cream. D.P.G.

## BREEDING

**231. Influence of Cell Concentration on Respiration Rate of Sperm.** C. F. WINCHESTER AND FRED F. MCKENZIE, Dept. Animal Husbandry,



Univ. Missouri, U.S.D.A. cooperating. Soc. Expt. Biol. and Med., Proc., 48: 648. 1941.

Respiration rate of ram and boar semen was measured by means of a modified Barcroft-Warburg respirometer. Sperm concentration was determined with a hemocytometer. An increase in concentration was found accompanied by a decrease in sperm respiration rate at sperm concentrations from 1 to 6 billion cells per cc. This effect was not invariably observed at concentrations of 0.2 to 1 billion sperm per cc. The effect of sperm concentration on sperm respiration rate did not appear to be due to changes in pH, diminished supply of O<sub>2</sub> to individual cells, an enzyme, or metabolic products.  
R.P.R.

232. Influence of Hydrogen Ion Concentration on Respiration Rate of Sperm. C. F. WINCHESTER AND FRED F. MCKENZIE, Dept. Animal Husbandry, Univ. Missouri, U.S.D.A. cooperating. Soc. Expt. Biol. and Med., Proc., 48: 654. 1941.

It was found that the hydrogen ion concentration of the media in which ram and boar sperm were suspended definitely influenced respiration rate. The optimum pH for respiration rate of boar semen was 7.2 to 7.3 and for ram semen it was 7.0 to 7.2. As the pH was raised or lowered from the optimum the respiration rate progressively declined. Unit changes in pH had significantly less influence on respiration rate of ram sperm than on that of boar semen.  
R.P.R.

## BUTTER

233. Value of Producer Interviews in Reducing Mold Mycelia in Butter. GAIL SMITH AND WALTER L. SLATTER, Ohio State Univ., Columbus. Natl. Butter and Cheese Jour., 33, No. 2: 12. 1942.

Sixty-two small cream producers on one cream route were divided into two groups, only one of which from April 23 to August 15 was interviewed and taught by personal visits at monthly intervals to use better methods of production. The cream from each group was collected and examined at weekly intervals and was churned separately. The interviewed group produced a higher percentage of first grade cream with an average Parson's test of 1.91, as compared with 2.37 for the other patrons. There was no significant difference in butter score and little difference in mold mycelia in the butter. It is not easy to improve quality of cream coming from small shippers.  
W.V.P.

234. Wapping and Packing Butter. E. G. HOOD, Dept. Agr., Ottawa, Canada. Natl. Butter and Cheese Jour., 32, No. 12: 8. 1941.

The quantity of butter degraded for surface defects, although not large, is of economic importance. Double-lining boxes with 40-pound or heavier

parchments minimizes but does not wholly overcome woody and other surface flavors; better results are obtained by using the double lining along with a casein-formalin treated box and a parchment head piece on the top surface. The casein-formalin treatment consists in applying two solutions simultaneously with a double-nozzle spray gun to the butter box or shook. Solution A is made in the proportions of 50 grams casein (from self-soured milk), 7.5 grams borax and 300 cc. water; solution B is 1.5 volumes of 45% formalin and 10 volumes of water. The two solutions are delivered simultaneously in the correct proportions over the whole treated surface. About one pound of casein solution (2 ounces of casein) and one-eighth pint of formalin solution are used per box. A new, improved one-piece liner made in part of aluminum has been developed but will not be available immediately; it can be sterilized with hot water. Surface bleach, bleached top or freezer scald can be prevented by treating parchment wraps with a 15% or stronger brine solution, rewetting the box liner, if necessary, with cold brine and by taking the butter leveler and finishing roller from a cold brine solution. High surface color caused by evaporation of moisture from surface layers can be cured by using a wrapper which gives a tight seal.

While improved methods of wrapping and packaging will assist in maintaining quality, high quality cream, careful methods of manufacture, controlled sanitary conditions, lack of delay between churn and storage, and low, uniform storage temperature are also necessary for keeping quality. Surface defects can be minimized by controlling the acidity of the cream to produce a pH of 6.8 to 7.2 in the butter; by reducing contamination of butter with heavy metals, especially copper; by avoiding exposure to light during manufacturing or packing; and by placing butter in storage at 0° F. as soon as possible after packing.

W.V.P.

235. **Distribution of *Pseudomonas putrefaciens*.** H. F. LONG AND B. W. HAMMER, Iowa Agr. Expt. Sta., Ames, Iowa. Jour. Bact., 41, No. 1: 100. 1941. (Abs. of paper presented at annual meeting of S. A. B.)

*Pseudomonas putrefaciens* causes a putrid or cheesy condition of salted butter. Materials were examined for the presence of the organism by direct smears on a special gelatin agar and by enrichment in litmus milk at 3° C. followed by smearing on the medium.

*Ps. putrefaciens* was isolated from raw, sweet milk and cream and from normal and putrid salted butter, but not from sour cream or highly ripened, unsalted butter, "where the sensitivity of the organism to acid may have resulted in its destruction." It was obtained from a sample of moist soil, but not from three samples of dry soil; from stream, lake and roadside water collected in various states; from creamery water supplies and from the

floors and sewers in dairy plants, particularly from sites that tended to remain moist.

In studies of dairy equipment, *Ps. putrefaciens* was obtained from parts of a butter printer in a plant that was having difficulty with putrid butter; from bolt heads, between staves and from the junction of staves and ends from three of four churns; and from the lining of a leaky milk vat immediately after the vat was taken out of service.

(See also JOUR. DAIRY SCI., Vol. 24: 921-924, 1941.)

D.P.G.

## CHEESE

236. **Italian Cheese Varieties.** J. C. MARQUARDT, N. Y. Agr. Expt. Sta., Geneva, N. Y. Natl. Butter and Cheese Jour., 33, No. 1: 10. 1942.

Much time and effort are required to produce and merchandise a foreign variety of cheese. The first requirement is to employ a qualified cheese-maker who knows how. Through the cooperation of Raphael Giolletti, Geneva, N. Y., and Hugo Bonetti, Caracas, Venezuela, translations have been made of procedures for making Caciocavallo, Provolone, Provole, and Percorino Romano. Some of these formulae have not been tried in the Geneva laboratory but are given as a guide to the best procedures outlined in Italian references.

Procedures for making American Provolone and Romano types are described briefly.

W.V.P.

237. **A New Method for Making Cheese.** C. C. FLORA, Virginia Polytechnic Inst., Blacksburg, Natl. Butter & Cheese Jour., 33, No. 1: 16. 1942.

Marketable cheese can be made more quickly by adding 5% starter immediately before the rennet, by diluting the whey with water to prevent whey acidity from exceeding 0.175% and by shortening all steps in processing. Water may be added after cutting the curd until the acidity is near 0.12%.

W.V.P.

238. **Manufacture of American Cheese from Pasteurized Milk.** H. L. WILSON, U. S. Dept. Agr., Washington, D. C., Natl. Butter and Cheese Jour., 33, No. 2: 18. 1942.

Experiences in laboratory and factory practice indicate that: the curing of pasteurized milk cheese may be inhibited by excessive acid development during making; pasteurization of milk gives a better and more uniform cheese; low grade milk injures the quality of pasteurized milk cheese; pasteurizing simplifies making and increases yield; and rate of acid development must be controlled. The schedule of manufacture is described. This type of cheese has proved well adapted to packaging in valved cans.

W.V.P.

- 239. Observations on the Composition of Cheddar Cheeses.** J. C. MARQUARDT AND M. W. YALE, N. Y. Agr. Expt. Sta., Geneva, N. Y. Natl. Butter and Cheese Jour., 32, No. 12: 16. 1941.

Analyses were made of cheese exhibited at the New York State fairs in 1940 and 1941. In 1940 some entries contained less salt than desirable; salt in the washed curd class was especially variable. In 1941 salt percentages were more uniform but generally tending too close to the lower limit. In 1941 about 7% of the samples contained more than the legal limit of moisture, but most lots had between 35.5 to 40.0%. A total of 319 samples of cheese selected at random in New York State and including cheese made in other states showed 22% with 36 to 38% moisture, 15.5% with less than 36% moisture, 32.5% with from 38 to 40% moisture, and 30% above the legal moisture limit; the average was well below 40%. Using the same 319 samples it was found that 50% contained less than the desirable minimum of 30% fat, about 30% had over 32% while the rest had from 30 to 32% fat.

W.V.P.

- 240. A Preliminary Study of the Effects of Varying Pitching Consistency and Rate of Scald on the Physical and Chemical Properties of Cheddar Cheese and on the Firmness of the Cheese as Judged by Cheese-makers, Bakers and Others.** G. W. SCOTT BLAIR, F. M. V. COPPEN AND D. V. DEARDEN. Natl. Inst. for Res. in Dairying, Univ. of Reading, Reading, England. Jour. Dairy Res., 12, No. 2: 170-177. 1941.

A preliminary experiment is described in which six cheese of the Cheddar type were made under conditions as similar as possible, except that the consistency of the curd at pitching, and in two cases the rate of scald, were varied. Chemical and rheological analyses, as well as measurements of moisture content and vapor pressure, were made on the cheese and also judgments of firmness under various conditions were given by cheesemakers, bakers and non-experts.

The firmness of the cheese appears to have been influenced by the consistency of the curd at the pitching point and by the rate of scald.

S.T.C.

- 241. Volatile Acids of Cheese III. Application of the Extraction Method.** E. R. HISCOX, J. HARRISON AND J. Z. WOLF. Natl. Inst. for Res. in Dairying, Univ. of Reading, England. Jour. Dairy Res., 12, No. 2: 155-169. 1941.

Details are given of an extraction method for the estimation of the volatile acids in cheese. Twenty grams of the cheese are shaken with 100 ml. of CO<sub>2</sub> free water at about 40° C. The mixture is centrifuged and cooled

to harden the fat which is then transferred to an ether solution. The supernatant liquid is poured into a flask and the residue washed twice more. The combined water washings are acidified to pH 2 with  $H_2SO_4$  and subjected to the usual steam distillation. The ether solutions of the fat were neutralized with NaOH, then washed six times with 50 ml. quantities of N/10 NaOH solution. After driving off the ether the combined washings are acidified to pH 2 and steam distilled. The water insoluble fraction was titrated separately from both distillations.

The total volatile acid as estimated by this extraction method was three to five times greater in Stilton and Gorgonzola cheese than estimated by direct steam distillation; and about twice as great in other cheese studied. For Stilton and Gorgonzola cheese the proportion of volatile acids in the distillate from the fat fraction was higher than that from the water fraction. The reverse was found in white cheeses such as cheddar and in blue cheese of the Roquefort type. This is taken to indicate that in the white cheeses such as cheddar, there is very little lipolysis and that water-soluble acetic acid (not derived from fat) constitutes the bulk of the volatile acids.

S.T.C.

## CHEMISTRY

242. Photochemical Studies of Rancidity: The Chlorophyll Value in Relation to Autoxidation. MAYNE R. COE, Agr. Chem. Res. Div. Bur. Agr. Chem. and Engin., U.S.D.A. Oil and Soap, 18, No. 11: 227. 1941.

Fresh oils fluoresce when placed under an ultraviolet lamp and the intensity of this fluorescence decreases with increased oxidation indicating that a loss of the reacting substance has taken place. When the acceptor molecules, or reacting substances, are progressively oxidized, or when an oil becomes rancid, they lose the property of quenching the fluorescence of chlorophyll. Advantage has been taken of this property of oils toward chlorophyll in following rancidity autoxidation, by titrating a given amount of chlorophyll with the oil under examination. The number of cubic centimeters of oil necessary to quench the red chlorophyll fluorescence is called the "Chlorophyll Value."

The chlorophyll value of an oil remains essentially the same as long as the oil is organoleptically sweet. The chlorophyll value test parallels very closely the results obtained by organoleptic methods used to evaluate the development of rancidity. The chlorophyll value test appears to enable one to detect and follow the very earliest stages in the process of rancidification, to obtain a quantitative knowledge of the state of oxidation and to make a comparison of the potential keeping qualities of any two or more oils or fats. Details of the method for determining the chlorophyll value of fats are given.

V.C.S.

- 243. The Fluorometric Determination of Riboflavin in Urine and Other Biological Fluids.** VICTOR A. NAJJAR, Dept. Ped., Johns Hopkins Univ. School of Med., Baltimore. *Jour. Biol. Chem.*, **141**, No. 2: 355. 1941.

A method for determining riboflavin in urine by measuring the fluorescence photometrically is given in detail. This procedure may be used for the determination of riboflavin in milk. The fat is first separated by high speed centrifugation and the proteins precipitated with trichloroacetic acid (2 cc. of skim milk plus 8 cc. of 10 per cent trichloroacetic acid). Five cubic centimeters of the filtrate are assayed for riboflavin by the direct method used for urine. V.C.S.

- 244. The Estimation of Lactose in Milk.** A. K. R. McDOWELL. Dairy Res. Inst. (N. Z.), Palmerston North, New Zealand. *Jour. Dairy Res.*, **12**, No. 2: 131-138. 1941.

The values for lactose content of milk as estimated by the following methods were found to show good agreement, and the author considers that they may be accepted as the true milk sugar content: 1. Direct volumetric copper reduction method using either unclarified milk or milk clarified and decalcified. Removal of the protein only resulted in low lactose values. 2. Polarimetric method using milk clarified with either zinc hydroxide or cadmium hydroxide. Clarification with acid mercuric nitrate or phosphotungstic acid gave high results. 3. Iodometric method using milk clarified with zinc hydroxide or cadmium hydroxide. Clarification with dialysed iron or phosphotungstic acid gave high results. 4. Chloramine-T method using milk clarified with dialysed iron, phosphotungstic acid, cadmium hydroxide or zinc hydroxide. S.T.C.

- 245. Studies on Ass's Milk. Composition.** C. P. ANANTAKRISHMAN. Dept. of Biochem., Indian Inst. of Science, Bangalore. *Jour. Dairy Res.*, **12**, No. 2: 119-130. 1941.

The author presents the following summary of his analysis of ass's milk:

The total solids of samples of ass's milk ranged from 7.14 to 8.50, and the fat from 0.54 to 0.71%.

The nitrogen distribution in ass's milk is: casein 39.5, albumin, 35.0, globulin 2.7 and non-protein nitrogen 22.8% of the total nitrogen. Ass's milk contains: casein 0.70, albumin 0.62 and globulin 0.07%. The total protein content is 1.39%. Ass's milk is therefore characterized by a low casein, a low globulin and a high albumin content.

The non-protein nitrogen consists of amino nitrogen 8.1, urea nitrogen 24.3 and uric acid 0.7 mg./100 ml. of milk. The urea content is twice that present in cow's milk.

The mean chloride and lactose contents of the milk samples are 0.037 and 6.1% respectively.

The average calcium and phosphorus contents of ass's milk are 0.081 and 0.059% respectively. Half the calcium is ionic, and half is in colloidal form.

The phosphorus distribution is: total acid soluble 84.0, acid soluble organic 38.5, easily hydrolysable ester 27.4, inorganic 46.0, and colloidal inorganic 23.0% of the total phosphorus. The ratio of  $\text{CaO} : \text{P}_2\text{O}_5$  is 1 : 1; 46% of the total phosphorus is in ester form; this is high when compared with only 12% in cow's milk; most of the phosphoric ester forms soluble barium salts, which is a distinguishing feature of ass's milk.

The total sulphur content is 15.8 mg./100 ml.

The fat has a penetrating odor and is colored orange-yellow. It has an iodine value of about 86, which is much higher than for human milk fat. The Reichert (9.5) and Kirschner values (5.7) are low.

In general, the composition of ass's milk resembles that of human rather than of cow's milk. S.T.C.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

246. **The Baking Industry—a Market for Dry Milk.** L. W. NOLTE, American Dry Milk Inst., Chicago, Ill. Natl. Butter and Cheese Jour., 32, No. 12: 14. 1941.

The baking industry is using three times as much milk solids today as 15 years ago; it uses more milk than any other single industry. Research in developing the use of dry milk in bread and in better methods of manufacture has made this possible. W.V.P.

257. **The Keeping Quality of Milk Powders. Part I. Addition of Antioxidants.** R. WAITE. The Hannah Dairy Res. Inst., Kirkhill, Ayr. Jour. Dairy Res., 12, No. 2: 178-183. 1941.

Hydroquinone in a concentration equivalent to 0.05% of the weight of fat (0.12% on the weight of powder), was found to be an effective antioxidant for the butterfat of spray dried whole milk powder, but produced an objectionable "metallic" flavor in the powder and reconstituted milk. Oat flour was much less efficient as an antioxidant but 0.25% added to the milk, preferably before condensing, increased the resistance of the resultant powder to the development of tallowiness by the equivalent of about 4 months at normal temperatures. Raising the concentration of oat flour to 0.5% doubled this increase but imparted a noticeable oat flavor to the product. S.T.C.

248. **The Effects of Additions of Dried Skim Milk and Dried Whey on the Baking Quality and Nutritive Properties of White Bread.**

K. M. HENRY, J. HOUSTON, S. K. KON AND J. POWELL, Natl. Inst. for Res. in Dairying, Univ. of Reading, Reading, England; and R. H. CARTER AND P. HALTON, Res. Assoc. of British Flour-Millers, St. Albans. *Jour. Dairy Res.* 12, No. 2: 184-212. 1941.

The authors present the following summary of their extensive work:

Experiments were carried out to study the effects on the quality and nutritive value of bread of the addition to white flour of roller-dried skim milk and roller-dried whey. Both samples were typical commercial products.

Additions of 2% of the dried milk could be made without any marked effect on loaf quality or on flavor. The addition of 4% or more definitely lowered the quality of the bread, the volume being smaller and the crumb more rubbery. As the content of milk was increased above 2% the flavor of the bread became increasingly distinctive and departed from the normal neutral flavor of water bread.

Up to 5% of dried whey could be added to the flour without any marked deterioration in the crumb of the bread, although with one flour this quantity decreased the volume by 16%. At this level, however, the whey imparted a distinct cheesy flavor to the bread.

Attention is drawn to the fact that the effects produced by the addition of dried milk or whey to bread can only be considered in relation to the particular sample used, since other workers have found that modifications in the method of manufacture considerably alter the value of the product as far as its use in bread is concerned. For this reason improved types of dried milk or whey might well lead to their greater use by the baking industry.

The addition of 2% milk solids doubled, 6% whey trebled and 6% milk quadrupled the calcium content of white bread. In hard-water districts a large part of the calcium of white bread is derived from tap water. When white bread was the sole source of calcium for young growing rats, their calcium intake was grossly subnormal. Owing to unavoidable metabolic losses only 60% of the ingested calcium was retained. The bread calcium was, however, well utilized and is probably as available as that of calcium acid phosphate and only slightly less so than milk calcium. Addition of dried skim milk or dried whey to the bread increased the percentage retention of calcium. This added calcium was almost completely retained.

A comparison of the biological values and true digestibilities of white bread, 2% milk bread and 6% milk bread by the method of Mitchell gave the following respective values: 44.7 and 90.9; 47.6 and 89.6; 49.7 and 88.9. A separate comparison of white bread, 2% white bread and 6% whey bread yielded similarly 47.4 and 92.9; 45.9 and 91.9; 47.4 and 88.9. At most, 15% of the bread proteins were derived from milk and no supplementary relation was detected at such low levels. Addition of 6% milk solids increased the "protein value" of bread by 25%.



Fluorimetric tests showed that the vitamin B<sub>1</sub> content rose from 1.0 µg./g. dry matter in white bread to 1.3 µg./g. in the 6% milk or whey loaves.

Riboflavin was similarly increased from 36 to 86–100 µg./g. It is possible that the full extent of the increase was not measured by the fluorimetric test.

Several experiments on rats in which the breads were fed as an exclusive diet showed that the marked beneficial effects of milk or whey additions were due not only to the increase in calcium but also to a large extent to the increase in riboflavin and other members of the vitamin B<sub>2</sub> complex.  
S.T.C.

## DISEASE

249. **Culture Media for Brucella.** GRACE P. KERBY AND ROYALL M. CALDER. Brucellosis Laboratory, Clayton Foundation for Research, Petroleum Building, Houston, Texas. Jour. Bact., 40, No. 5: 637. 1940.

In an attempt to secure a culture medium that is reliable for clinical use a large number of materials were investigated for their effect as growth-promoting factors for *Brucella* when added to the basic medium, Bacto-Tryptose broth. Ratings were made on the basis of a standard comparison of each series with tryptose broth, as prepared in uniform lots by "Difco."

Milk medium containing 2 per cent Bacto-Tryptose, 0.5 per cent sodium chloride, 1:700,000 crystal violet, made up with fresh, whole, Grade A pasteurized milk and sterilized by tyndallization, showed earlier and more abundant growth than that obtained in a tryptose broth control medium. Also, growth was positive in the milk medium when the inoculum had been reduced beyond the point where *Brucella* could be recovered from tryptose broth. The use of skimmed milk, homogenized milk, powdered milk, certified raw milk, or heavy cream as a base in this medium was not satisfactory. Incorporation of a 50 per cent liver-infusion base into the milk medium seemed to have a slightly favorable effect, but the results were not convincing. Media made with peptonized or trypsinized milk base were inferior to tryptose broth. The pasteurized whole milk medium was not dependable due to variation in the lots of milk used.

Various sodium caseinate media were studied in the series. The one most effective in growing *Br. abortus* 456 consisted of 2 per cent sodium caseinate, 2 per cent Bacto-Tryptose, 0.1 per cent Bacto agar, 0.5 per cent sodium chloride, and 1:700,000 crystal violet. Preparation of sodium caseinate medium can be expected to result in uniform lots of media but the growth of only *Br. abortus* 456 was enhanced in a series including 1 recently isolated *Br. melitensis* strain, 4 old *Br. melitensis* strains, 1 old porcine strain, 2 recently isolated *Br. abortus* strains and 4 old *Br. abortus* strains.

The value of sodium caseinate medium in routine culture work is questionable.

Addition of cod liver oil (16 per cent) resulted in inhibition of growth in milk media and in tryptose broth. Various tomato juice media failed to support growth of the organism. Addition of carrot extracts to tryptose broth resulted in slight inhibition of growth.

Attempts to enrich tryptose media by addition of vitamins A, B<sub>1</sub> and B<sub>2</sub> in the form of Embo products were unsuccessful, actually inhibitory, as were Hormodin A, and anterior pituitary extract. Spleen-infusion agar did not prove superior to tryptose agar. Glutathione yeast extract, gelatin, whey and *Bacillus subtilis* extracts did not improve tryptose media. Slight inhibition was noted in media containing Bacto Asparagine, Bacto Malt Extract, bone meal extracts, bone marrow extracts, purified corn phosphatid, and soy bean extracts. Sodium sulfite, 0.05 per cent in tryptose broth, slightly inhibited *Brucella*. Also unsatisfactory were liver-infusion, the Burky modification of Huntoon's hormone medium, and Brewer's sodium thioglycollate medium. D.P.G.

250. The Relationship of Methods of Bacteriological Examination to the Eradication and Control of Mastitis (*Streptococcus Agalactiae*). I. Use of Enrichment Technique in Revealing Streptococcal Infections of the Cow's Udder. II. *Streptococcus agalactiae* Infections in Heifers. A. T. R. MATTICK, P. M. F. SHATTOCK AND M. MOREIRA JACOB. Natl. Inst. for Res. in Dairying, Univ. of Reading, Reading, England. Jour. Dairy Res., 12, No. 2: 139-154. 1941.

In the course of attempts on a field scale to eradicate or control *Str. agalactiae* infections, comparisons of the efficiency of several bacteriological methods were made. An enrichment method in which 9.5 ml. of milk plus 0.5 ml. of an alcohol water solution of brom-cresolpurple to give a final concentration of 0.025 per cent was incubated at 37° C. (98.6° F.) for 24 hours, prior to streaking on aesculin crystal violet blood agar plates, was found to detect many lightly infected cases which were reported as negative using sodium azide broth or the plating on Edward's medium of the centrifuged deposit. Increasing the frequency of examination was shown to increase materially the number of positive cases discovered.

*Streptococcus agalactiae* was found to be present in 21 per cent of the animals in their first lactation and 4.5 per cent of heifers were found to harbor this organism while in isolation before joining the herd. The authors suggest the probability that if more stringent methods were devised and examinations at very short intervals made, the organism could be demonstrated in the milk of a high proportion of milking cattle. The "carrier" state may thus be widespread. S.T.C.

## FEEDS AND FEEDING

- 251. Magnesium Studies in Calves. II. The Effect of Magnesium Salts and Various Natural Feeds upon the Magnesium Content of the Blood.** C. F. HUFFMAN, C. L. CONLEY, E. C. LIGHTFOOT AND C. W. DUNCAN, Dairy Husbandry Dept. and Agr. Expt. Sta., Michigan State College, East Lansing, Mich. *Jour. Nutr.*, 22: 609-620. 1941.

Calves fed a basal ration consisting of whole milk supplemented with iron, copper, and manganese, with or without the addition of starch or other similar carbohydrates always resulted in subnormal blood plasma magnesium values. Normal plasma magnesium levels resulted by the addition of magnesium in sufficient quantities to bring the intake up to 30 to 40 mg. of magnesium per kilogram of body weight. When either corn or alfalfa hay was fed as supplements to the basal ration, a total intake of 12 to 15 mg. of magnesium per kilogram of body weight was sufficient to maintain normal levels. When sufficient magnesium oxide was fed with corn gluten meal so that the magnesium intake was equal to that when corn was fed 12 to 15 mg. of magnesium per kilogram of body weight also was sufficient to maintain normal blood plasma magnesium values. The magnesium in alfalfa ash was not used efficiently. These data indicate the utilization of magnesium by growing calves is more efficient when magnesium is furnished by natural feed than when supplied by magnesium salts.

C.F.H.

- 252. The Utilization by Calves of the Energy Contained in Balanced Rations Composed of Combinations of Different Feeds.** H. H. MITCHELL AND T. S. HAMILTON, Anim. Nutr. Div., Univ. Illinois, Urbana, Ill. *Jour. Nutr.*, 22: 541-552. 1941.

Eight Shorthorn calves were fed four different rations containing similar proportions of the various classes of nutrients distinguished by approximate chemical analysis, and to be adequate in all of essential nutrients at two different levels of nutrition. The metabolism and respiration experiments on these steers indicated that the metabolizable energy of the various rations is equally utilized for maintenance and for body increase.

The percentage net availability of the metabolizable energy was quite similar for the four experimental rations at the lower plane of nutrition and also on the higher plane of nutrition.

C.F.H.

- 253. Biennial Reviews of the Progress of Dairy Science. Section A. Physiology of Dairy Cattle. II. Nutrition.** ANONYMOUS. Sec. F. Milk-Borne Diseases. *Jour. Dairy Res.*, 12, No. 2: 213-240. 1941.

Recent literature on dairy cattle nutrition is reviewed under the follow-

ing headings: Feeding standards, the feeding of calves, utilization of non protein nitrogen, mineral metabolism and vitamins. 152 references. Literature on milk borne diseases is reviewed under the headings, epidemiology, streptococcal infections, staphylococcal food poisoning, tuberculosis, salmonella infections, dysentery, brucella infections, virus infections and miscellaneous infections. There are 71 references. S.T.C.

## FOOD VALUE AND DAIRY PRODUCTS

- 254. Vitamin A and C in Cow's Milk, with a Note on the Synthesis of Vitamin C in Bovines.** S. N. RAY, KARAM CHAND AND K. GOVIND RAU. Imperial Vet. Res. Inst., Mucktesar, India. Jour. Dairy Res., 12, No. 2: 109-118. 1941.

The mean carotene and vitamin A content of 16 samples of milk from different breeds of cows was 71 ( $\pm 32.03$ ) Moore's yellow units and 115.6 ( $\pm 35.46$ ) Moore's blue units, respectively per 100 ml. milk. The mean value for reduced ascorbic acid in the milk from 13 cows was 1.94 ( $\pm 0.35$ ). No great individual variation in ascorbic acid was found.

A vitamin C free ration was prepared from a grain mixture and wheat straw in the following manner: The concentrate and roughage mixtures were made alkaline by moistening with caustic soda solution and autoclaved at 120° C. for two hours. The autoclaved mass was kept over night, then dried in a hot-air oven at 120° C. and then crushed in a mill. Analysis showed a reduction in the ascorbic acid content of the grain mixture from 1.65 mg. to 0.45 mg./100 mg., and in the wheat straw from 0.85 to 0.28 mg./100 g. Since these values could not be decreased by further heating, it was assumed the residual reducing substances were not ascorbic acid. The concentrations of ascorbic acid in the blood plasma of calves fed on the heated ration was not lower than that of calves fed on the unheated ration, showing synthesis of vitamin C in the animal body.

Unsuccessful attempts were made to produce vitamin C in vitro by growing bacteria isolated from various parts of the alimentary canal on media prepared from ingesta taken from the regions from which they were isolated. S.T.C.

## ICE CREAM

- 255. Some Newer Ice Cream Stabilizers and Their Functions.** ALLAN LEIGHTON, Div. Dairy Res. Labs., U. S. Bur. Dairy Indus., Washington, D. C. Ice Cream Trade Jour., 37, No. 12: 12. 1941.

A vegetable stabilizer of the alginate type and a mixture of mono-glyceride and gelatin were compared with gelatin as stabilizers for ice cream. The newer type stabilizers shortened the whipping time and increased the maximum amount of overrun obtainable. Evidence was pre-

sented to show that more water is present in the form of ice in the ice cream stabilized with alginate and monoglyceride gelatin alone, a condition which is associated with small ice crystals and a smooth texture. It was concluded that these new type stabilizers were capable of producing ice cream of excellent quality and also capable of obviating difficulties encountered in the whipping process incident to freezing. W.H.M.

- 256. Reducing Power Costs.** C. T. BAKER, Atlanta, Ga. *Ice Cream Rev.*, 24, No. 8: 43. 1941.

The cause for high power bills should be traced and corrections made. The situation found in one ice cream plant is discussed. J.H.E.

- 257. Variegated Ice Cream.** ANONYMOUS. *Ice Cream Rev.*, 24, No. 8: 42. 1941.

The variegated or ribbon type of ice cream is apparently here to stay, the most successful flavors being chocolate, strawberry, raspberry and butterscotch. A manufacturing difficulty is the settling of heavy flavors to the bottom of the can. Prevention lies in freezing the ice cream stiffer and using less sugar in the syrup portion. Iciness can be overcome by the use of more pectin and chilling the syrup down to 30° F. before adding. J.H.E.

- 258. Superheated Condensed Milk in Ice Cream.** J. H. ERB, Ohio State Univ., Columbus, O. *Ice Cream Rev.*, 24, No. 8: 56. 1941.

The "superheated" process applied to ordinary whole or skim condensed milk is a method of obtaining greater water binding power from milk proteins. Ice cream made from superheated milk is more resistant in body and smoother in texture. Two methods for making superheated condensed milk are given, as well as factors influencing time required to superheat. J.H.E.

- 259. Ice Cream Tarts.** VINCENT M. RABUFFO, Editor. *Ice Cream Trade Jour.*, 38, No. 1: 10. 1942.

Many ice cream manufacturers are stimulating winter sales by offering a new item known as ice cream tarts.

Each tart contains 3½ to 4 fluid ounces of ice cream and about an ounce of fruit, or about 4 to the pint or 32 to the gallon of ice cream. The paper tart dishes are 3½" in diameter at the top and 1½" deep. Fruits used for filling are chilled to about the same temperature as the ice cream, and after placing in the dish it is decorated with a ring of whipped cream.

Raspberries, cherries, pineapple, strawberries, and peaches may be used in tarts. A box of 4 retails at 35 to 38 cents, or they may be sold individually from 10 to 15 cents. W.H.M.

**260. Research in Ice Cream During 1941.** W. J. CORBETT, Univ. Illinois, Urbana, Ill. Ice Cream Trade Jour., 37, No. 12: 14. 1941.

Important research in the field of ice cream making was reviewed and attention directed to the following findings:

1. The flavor and body and texture of ice cream were not affected by type of frozen condensed milk used nor the length of time it was held frozen prior to using.

2. Heating cream to 170° F. and the addition of 2% oat flour (on fat basis) prevented or reduced the development of oxidized flavor during the storage period. The addition of 10% sugar to cream before freezing did not prevent development of off flavor but it did aid in defrosting and reduced oiling off. Acid reduction to 0.10% in mixes containing frozen cream increased the whipping ability.

3. A method for making and using soluble casein in ice cream indicated that up to 40% of the serum solids of the mix could be supplied from this source. Higher amounts resulted in a curd-like flavor and gummy body. A marked increase in whipping ability was observed when the mixes contained the casein-gel.

4. Work by several investigators working with the various sweeteners made from corn indicate that 25 to 33% of the sucrose on a sweetness basis can be replaced by the corn sugars without any injury to the quality of the ice cream. In some instances an improvement in body and texture resulted from the substitutions.

5. Improvement in whipping properties of the mix and in the body and texture of the ice cream resulted when 0.4% of a homogenous mixture of 0.06% monoglyceride and 0.30% gelatin was used as a stabilizer.

6. The addition of calcium and magnesium alkalies to ice cream mixes seems to have some merit in controlling mix acidity.

7. Vaccination method of pasteurization of ice cream mixes resulted in some improvement in flavor. The mixes were more resistant to the development of oxidized flavor and less flavor was required in the ice cream. This method was also efficient in reducing bacterial counts.

8. Sodium alginate and locust bean gum exerted a more noticeable protective action than gelatin and sugar in retarding bacterial destruction when mixes were pasteurized at 143° F.

9. Ice cream comparable to that from a mix homogenized at 1000 to 1500 pounds pressure was produced from a mix homogenized at 500 pounds on a rotary machine, but it was inferior to that produced when 2000 to 3000 pounds pressure was used.

10. The higher the homogenization pressure and the oftener a mix is homogenized the wetter the ice cream appears when drawn from the freezer. Higher pressures resulted in a more rapid and smoother melt-down and some improvement in body.

11. A suitable syrup to use in the manufacture of variegated ice cream should have a desirable color, contain a satisfactory stabilizer (1% pectin), have a sugar content of approximately 40%, and have a proper acidity (about 1%).

12. A simplified tester and a procedure for determination of total solids in ice cream mix has been developed by Harmon and Renner at the Texas Technological Institute, Lubbock, Texas.

13. A method has also been developed to detect the presence of sodium alginate in dairy products by Shroeder and Racidot (*Journal Indus. and Engin. Chem., Analyt. Ed.*, 13, No. 3: 165. 1941). W.H.M.

261. **Fancy Form "Fotos."** ANONYMOUS. *Ice Cream Trade Jour.*, 38, No. 1: 14. 1942.

C. Everett Sells, fancy form technician for Moores and Ross, Columbus, Ohio, has developed a method of transferring photographs or emblems to individual slices of ice cream.

It is done by printing the picture or emblem on a piece of sensitized silk, then spraying certified food color through this screen. Bricks of ice cream are cut into eight portions and then dipped in a mixture of whipped cream and soft ice cream which is thinned to about the consistency of chocolate coating to give a smooth surface on which to stencil the image.

The cost of making the stencil is about double the price of an individual mould. W.H.M.

262. **Mix Stabilizers.** P. H. TRACY, Univ. Illinois, Urbana, Ill. *Ice Cream Rev.*, 24, No. 8: 44. 1941.

This article is a review of the common ice cream stabilizers and the effect of each on mix and ice cream properties. Acid-type gelatines were found superior to alkaline gelatines. High Bloom test (275 grams) gelatines were found to give better results than low. One tenth to 0.30% of locust bean was found to have a desirable effect upon the body of ice cream. However, the ice cream showed poor melting characteristics with this stabilizer. A combination of locust bean with gelatin gave improved melt-down characteristics.

Addition of pectin to the mix will produce some stabilizing results but it is desirable to use it in combination with gelatin.

When comparing 0.30% sodium alginate and 0.35% gelatin the body scores of the ice cream were comparable and except in counter freezers there was little difference in whipping qualities.

As little as 0.10% Irish moss produced body results practically comparable with those produced with 0.35% gelatin (215 Bloom). The body was slightly more crumbly than gelatine stabilized ice cream.

Gum tragacanth and gum karaya are used successfully in ices and sherbets. J.H.E.

**263. Diabetic Ice Cream.** ANONYMOUS. *Ice Cream Rev.*, 24, No. 8: 36. 1941.

The experience of a company producing an ice cream suitable for people suffering from diabetes is cited. The use of 0.025% saccharin is used in place of 16% sugar. The mix should have about 38% total solids. Since milk concentrates cannot be used because of their high protein content, glycerol can be used to build up solids.

The fact that glycerol and saccharin are used should be printed on the containers in which the ice cream is sold. Diabetic patients are prescribed a diet low in carbohydrates, low in proteins and high in fats. J.H.E.

## MILK

**264. The Effect of the Two Quart Bottle and Gallon Jug on Plant Operations.** GEORGE NINOW, The Akron Pure Milk Co., Akron, Ohio. *Internatl. Assoc. Milk Dealers, Assoc. Bul.*, 31: 121-124. 1941.

Variations in the style of multiple quart containers are great. Capping presents problems and trouble has been expressed by some with corrosion of handles by strong alkali or chlorine. Much trouble has been due to the earlier equipment for filling and washing these containers not being suited to large scale operations. Due to greater value very few gallon containers find their way to rubbish piles. The decrease in efficiency due to a shorter run on small units, the unavoidable added labor costs due to another inventory item, and other considerations do not make it evident that there should be any large spread between the price of milk in quart and larger containers.

E.F.G.

**265. Plant Layout for Paper Container Equipment.** J. H. FORSLEW, Bowman Dairy Co., Chicago, Ill. *Internatl. Assoc. Milk Dealers, Assoc. Bul.*, 34: 117-120. 1941.

This discussion relates experience with the American Can Co. preformed milk container.

The handling of empty containers instead of being a simple operation is a technical and exacting operation not to be entered into without careful consideration and due regard for clean sanitary storage space and surroundings, proper handling and conveying equipment, and infinite care and control required at all times to maintain correct operating conditions. The superintendent should give his personal supervision to unloading and handling until the men have learned to properly handle the containers as the tendency is to treat them like glass bottles. Besides protection from con-



tamination and smoke it is important that containers do not get too warm as paraffin will soften and adhere to working parts of conveyor and filler. Filler and conveyor should be washed down every hour or two with hot water to remove paraffin accumulations. Chain conveyors are preferred for the incoming cases of empty bottles but belt conveyors are used to carry bottles to the filler. Inspection of both sides of the bottles by the operator is accomplished by means of properly placed mirrors. It is important that milk be as cold as possible as bottles are more rigid at the lower temperature. A metal canopy painted black inside with a tubular light beneath permits the operator to see how full the bottle is. Vans are packed solid with a cake of ice in each end. E.F.G.

- 266. Plant Problems in Connection with the 48 mm. Bottle.** Ross J. WINNING, Sheffield Farms Co., Inc., New York, New York. Internatl. Assoc. Milk Dealers, Assoc. Bul., 34: 114-116. 1941.

The larger roll at the top of the old 56 mm. bottle presented a greater illusion as to fullness than does the 48 mm. bottle with its smaller roll. Narrowness of neck on the latter bottle accentuates the lack of fullness. Incorporation of air in the milk and centering the bottle are more trouble problems in the case of the narrower neck bottle. The fill should be more precise with the new bottle. Besides difficulty in properly centering and seating the cap more trouble with milk seepage is experienced due to the smaller allowance for expansion as the milk becomes warmer. In the bottle washer centering with regard to jets must be more carefully done. The new bottle has some advantage in trippage. E.F.G.

- 267. Simplified Designs for Glass Milk Bottles, Standardization of Glass Milk Bottles.** V. L. HALL, Glass Container Assoc., New York City. Internatl. Assoc. Milk Dealers, Assoc. Bul., 34: 107-113. 1941.

The first move toward standardization of milk bottles took place in 1926 and resulted in Simplified Practice Recommendation No. 10 issued by the Department of Commerce. In spite of this, by 1937 there were 33 distinct quart shapes. A great advantage of standardization of design is the simplification of many equipment problems. Standardization will also be a great aid in offsetting rising material and labor costs since the standardized container can be produced more cheaply.

It is recommended that: (1) Elimination of all shapes except the Economy (17½ oz.) and the three standard shapes (22 oz.) for quarts and the Standard two-quart. (These are illustrated.) (2) Elimination of all but seven types and sizes of finishes. (These are named.) (3) Elimination of all but four dimensions of cap seats. (4) Avoidance of neck decorations. (5) Dairies using non-standard bottles be supplied but that an early change to a standard type be considered.

Adherence to the above program would provide 35 combinations of shapes with various diameter cap seats with or without lugs and bumper rolls for the quart and two quart sizes. This is about one-fourth of the combinations permitted by present standards and an even smaller fraction of the variety of bottle specifications now being ordered by dairymen. In the article drawings illustrate the various type bottles and finishes.

E.F.G.

**268. Policy Considerations in the Administration of State Milk Control Acts.** W. J. KUHRT, California State Dept. Agr., Sacramento, Calif. Internatl. Assoc. Milk Dealers, Assoc. Bul., 34: 143-151. 1941.

The first state control act was passed in 1935 and by 1941 more than 88% of the milk consumed in California came under state control. Emphasis in the control measures was placed upon correction of chronic unsatisfactory conditions and practices in the market rather than mere alleviation of the depression effects. The essential objectives of the control act are: to provide specific procedures for determining producer prices and distributor margins including the differential between fluid milk prices and manufacturing milk prices, and proper formulation of contracts. Authority to fix margins has resulted in more efficient delivery. Facts are obtained by detailed exhaustive surveys of both production and distribution and the results are available to all producers and distributors. Prompt modification of prices is provided for by an automatic change of 5 cents per pound of fat to the producer and  $\frac{1}{2}$  cent distributor price change as the cost of feed and the manufacturing milk price changes. A levy of 2 mills per pound of milk fat from both producers and distributors provides about \$300,000 per year to cover expenditures divided about half for fact finding and half for enforcement.

The California Supreme Court upheld the constitutionality of the act in 1940. In California employees are under civil service regulations and their positions are secure from undue influences of pressure groups. No program is undertaken until a petition signed by at least 65% of the producers who produce at least 65% of the milk has been received. The law is administered "in the public interest" and it is especially important that this fact is kept constantly before the public if public confidence is to be retained.

Some of the results of the milk control act are establishment of 1 cent differential between store and home delivery, a half cent higher charge to the store for milk in paper containers, and the establishment of prices of milk sold to federal agencies. Consumption of milk is being encouraged through an educational program, through a lower multiple quart container price and by a special price on a 3.65% fat milk in a half gallon container.

E.F.G.

- 269. Fluid Milk Markets and the Lend-Lease Program.** P. L. MILLER, U.S.D.A., Washington, D. C. Internatl. Assoc. Milk Dealers, Assoc. Bul., 34: 152-157. 1941.

The attempt to increase the production of butter and cheese by 30-40% and dry skim milk by 60% has resulted in a price for fat in milk used for these products usually above that paid for fat in farm separated cream. As the price for manufacturing milk increases, the question arises as to whether the price of fluid milk should be as high relative to manufacturing milk as it was before the lend-lease program. It is the view of the Department of Agriculture that increased supplies of dairy products are also essential for our own people in building up our national defense. Adequate supplies of fluid milk are needed and as the price structure has seemed to be inadequate to secure those supplies upward adjustments of fluid milk prices are in order. Requests for hearings on amendments to fluid milk marketing order have been acted upon as promptly as possible. Warranted price increases in fluid milk are not considered inflationary. E.F.G.

- 270. Seasonal Variations in Thermoduric Organisms and Methods of Control.** H. MACY AND J. A. EREKSON. Internatl. Assoc. Milk Dealers, Assoc. Bul., 34: 127-135. 1941.

Standard plate counts were made before and after laboratory pasteurization on 7,562 samples of milk from the St. Paul—Minneapolis milk shed during four seasons of the year. Field inspections were also made. Thermoduric organisms varied in number in relation to season and quality of raw milk. The thermo-tolerant organisms may be found in low as well as high count raw milk but a higher proportion of the poorer quality milk will be difficult to pasteurize satisfactorily regardless of the season. Percentage reduction is generally greater in high count milk although the number of thermoduric bacteria may be greater than in low count milk. In summer 40.5% of the samples showed less than 5,000 bacteria per ml. after pasteurization, while in winter the proportion below this figure increased to 80.1. These data indicate larger numbers of thermoduric bacteria in the summer milk usually due either to contamination of utensils or faulty cooling. Very high counts can usually be traced to improperly washed utensils or poor can washing. Laboratory pasteurization or individual producers' milk will locate the samples giving trouble and field inspection can usually eliminate the trouble. Careful producers will clean and sterilize a plant-washed can before using it if it has been allowed to stand some time under warm humid conditions. Milking machines are the most prolific single source of contamination; they were used on 41% of the farms having trouble and were found to be the responsible factor in every case. Wherever milkstone is present there will likely be trouble with thermodurics. Split seams, cracks,

crevices, broken solder or rusty spots or imperfections in rubber parts invite trouble. E.F.G.

- 271. Put Your Taste Buds to Work on Your Flavor Problems.** K. G. WECKEL, Univ. Wisconsin, Madison, Wis. Internal. Assoc. Milk Dealers, Assoc. Bul., 34: 136-140. 1941.

The bland flavor of milk probably accounts for the fact that it can be used with regularity in considerable quantities. However, if an unnatural flavor appears in the milk, many people quickly object to it and probably recognize such a flavor more readily than in most other foods. A small minority may dislike certain flavors such as oxidized, while the majority of milk users considers such flavors normal. Instead of waiting for flavor complaints by customers it is suggested that systematic flavor examinations of the product be made by one or two persons who have made an attempt to develop ability along this line. Education in detection and identification of flavors is proceeding in many consumer groups. Attention is called to work by Professor Wallenfeldt with agricultural and home economics teachers of Wisconsin to test ability to identify (a) by tasting solutions of salt, sugar, acid, and quinine and (b) by odor such substances as vanilla, orange, mint, lemon, maple, coffee, tobacco, fresh silage, etc. Men tend to excel in identifying solutions by taste but women excel in identifying odors.

E.F.G.

- 272. The Effect of Pasteurization on Some Constituents and Properties of Goats' Milk.** H. S. HALLER, C. J. BABCOCK, N. R. ELLIS, U.S.D.A. Tech. Bul., 800. 1941.

The effect of pasteurization on the solubility of calcium and phosphorus, on the denaturation of proteins, and on the ascorbic acid content of goats' milk was found to be comparable to the effect of pasteurization upon the same constituents of cow's milk, as reported in the literature.

Curd tension was reduced considerably by the holder method of pasteurization but only slightly by high temperature pasteurization. Ascorbic acid was reduced 33 to 45% by the holder method but there was no effect from the high temperature method of pasteurizing.

The phosphatase enzyme in goats' milk was destroyed after 5 minutes heating at 143° F. making the phosphatase test as now conducted not applicable to goats' milk. P.H.T.

- 273. Sour Cream—How to Prepare and Use It at Home.** Anonymous. U.S.D.A., Leaflet No. 213.

It is recommended that the cream be pasteurized before innoculating with commercial buttermilk. Directions for doing this in the home are

given. A number of recipes for the use of sour cream is included in this pamphlet. P.H.T.

## PHYSIOLOGY

- 274. Uterine Distention and Lactation in the Rat.** R. R. GREENE, Dept. Physiology and Pharmacology, Northwestern Univ. *Endocrinology*, 29: 1026. 1941.

Ten to 15 cc. of paraffin were injected into the uteri of 15 rats within 24 hours of delivery. Within 48 hours 3 rats and their young were dead. Of the remaining 12 animals 6 successfully raised their litters although there was little or no weight gain of the young in the first 3 to 5 days. The young of the remaining 6 rats were dead within 24 hours to 6 days after the operation although a small amount of milk could be expressed from the mammary glands of the mothers. It was concluded that uterine distention *per se* is unable to prevent lactation. R.P.R.

- 275. Failure of Steroid Hormones to Induce Mammary Growth in Hypophysectomized Rats.** SAMUEL L. LEONARD AND RALPH P. REECE, Dept. of Zoology and Dairy Husbandry, Rutgers Univ. *Endocrinology*, 30: 32. 1942.

Desoxycorticosterone, testosterone and estrogen, either alone or in combination failed to induce new growth in the mammary glands of hypophysectomized rats. Testosterone seemed to slow the rate of mammary gland involution which normally follows hypophysectomy. The administration of estrogen directly on the skin over the mammary gland of hypophysectomized rats was without effect although in the normal or partially hypophysectomized rat new growth was stimulated. The results of the experiments were believed to further the concept that pituitary mammogen is essential for steroid hormone effects on the mammary glands of hypophysectomized rats. R.P.R.

- 276. Mammary Gland Development with Mammogen I in the Castrate and the Hypophysectomized Rat.** A. A. LEWIS, E. T. GOMEZ AND C. W. TURNER, Dept. Dairy Husbandry, University of Missouri and Bureau Dairy Industry, U.S.D.A. *Endocrinology*, 30: 37. 1942.

The injection of a dosage of 0.02 to 1.6 mouse units of a lipid mammogen I extract of pregnant-cattle anterior pituitary glands over a 6 day period produced no mammary growth in castrated male rats. Positive results, were obtained following either longer periods of treatment or increased dosage. Of sixteen 25- to 44-day-old male and female hypophysectomized rats treated with mammogen I for 7 to 10 days 15 showed evidence of mammary growth at autopsy. The weights of the thyroids, ovaries, and uteri of treated hypoph-

ysectomized female rats were similar to those of untreated hypophysectomized female rats. Adrenal weights of treated rats were significantly less than those of untreated rats. R.P.R.

- 277. Mammary Growth in Hypophysectomized Male Mice Receiving Estrogen and Prolactin.** W. U. GARDNER AND ABRAHAM WHITE, Dept. Anatomy and Physiol. Chem., Yale Univ. Soc. Expt. Biol. and Med., Proc., 48: 590. 1941.

Hybrid male mice were hypophysectomized at 4 to 8 weeks of age and from 3 to 47 days later received either the lactogenic hormone alone or in conjunction with estrogen. The lactogenic hormone was administered intraperitoneally and the estrogen subcutaneously on alternate days. Completeness of operation was checked by examination of serial sections of the sella. Of 11 mice which were completely hypophysectomized and which received a purified prolactin preparation 3 of them showed mammary growth. Of 13 completely hypophysectomized mice injected with the lactogenic hormone plus an estrogen all showed mammary growth. R.P.R.

- 278. Effect of Various Dietary Supplements on Growth and Lactation in the Albino Mouse.** ZELDA B. BALL AND RICHARD H. BARNES, Dept. Physiol., Univ. Minnesota. Soc. Expt. Biol. and Med., Proc., 48: 692. 1941.

It was found that purified diets containing 8% yeast were not adequate for lactation in mice. Dehydrated grass and wheat bran effectively increased lactation but the addition of these two substances to a basal purified diet, either alone or in combination, did not provide for as good lactation as did a stock commercial diet. The lactation-promoting effect of dehydrated grass and wheat bran was found not to be due to an increase of thiamin, riboflavin, pantothenic acid, factor W, or inositol in the diet. R.P.R.

- 279. The Gonad-Stimulating Potency of the Pituitary of Hypothyroid Young Male Rats.** KATHYRN F. STEIN AND MARGARET LISLE, Dept. Zoology, Mt. Holyoke College. Endocrinology, 30: 16. 1942.

Fifty-five 21- to 28-day-old male rats were thyroidectomized and after 21 to 50 days their pituitaries were injected into immature female mice 18 to 20 days of age. The recipients were given 1 to 3 pituitary glands and they were killed on either the 4th or 5th day after the first injection. Thyroidectomy invariably elicited an increase in pituitary weight and the presented data indicated a decrease in gonad-stimulating potency of the pituitary following thyroidectomy. R.P.R.

- 280. Gonadotropic Hormone in A P of Male and Female Rabbits during Growth.** A. J. BERGMAN AND C. W. TURNER, Dept. of Dairy Husbandry, University of Missouri. *Endocrinology*, 30: 11. 1942.

The amount of gonadotropic hormone in the pituitaries of groups of male and female New Zealand White Rabbits of increasing body weight was determined by injecting the pituitary tissue into male chicks and weighing their testes at the end of the injection period. There was a gradual rise in hormone concentration which reached a maximum in a group of females weighing 1500 gm. and in the males weighing 2500 gm. There was a notable decrease in hormone concentration of pituitaries collected from a group of non-breeding female rabbits weighing around 4500 gm. The hormone concentration in the AP of sexually mature males averaged about 70 per cent above that of females of comparable weight. R.P.R.

- 281. Influence of Environmental Temperature on Growth of Mammary Lobule-Alveolar System.** JOHN P. MIXNER AND CHARLES W. TURNER. *Soc. Exp. Biol. and Med., Proc.*, 48: 443. 1941.

Ovariectomized virgin mice kept at a high environmental temperature had a decreased ability to respond to progesterone and estrone injections as judged by mammary lobule-alveolar growth. High temperature did not inhibit mammary response in mice injected with a pituitary extract containing the lobule-alveolar growth factor in conjunction with estrone. It was concluded that the anterior pituitary of animals kept at a high environmental temperature has a decreased ability to respond to the stimuli of progesterone and estrone as indicated by the secretion of the lobule-alveolar growth factor. R.P.R.

- 282. Influence of Lactogenic Preparations on Mammary Glands and Time of Vaginal Opening in Young Rats.** WILLIAM R. LYONS, M. E. SIMPSON AND H. M. EVANS, Inst. Expt. Biol. and Div. Anat., Univ. California. *Soc. Expt. Biol. and Med., Proc.*, 48: 634. 1941.

The subcutaneous administration of lactogenic hormone in doses from 0.5 to 2.0 mg. daily to 21-day-old rats did not delay vaginal opening and in some cases may have advanced it. The continued injection of the same doses of hormone produced continuous vaginal mucification (two weeks) and lobule-alveolar development of the mammary glands. R.P.R.

- 283. Effect of Pseudopregnancy on Mammary Carcinoma Incidence in Mice of the A Stock.** L. W. LAW, ROSCOE B. JACKSON, Memorial Lab., Bar Harbor, Maine. *Soc. Expt. Biol. and Med., Proc.*, 48: 486. 1941.

A definite increase in the mammary carcinoma incidence occurred in

female mice of a tumorous strain following pseudopregnancy induced by mating with vasectomized males. Eleven or 25.6% of 43 experimental mice developed breast adenocarcinomas whereas it had been previously shown that the incidence of carcinoma in virgin mice was only 4.9%. R.P.R.

- 284. Quantitative Study of the Effect of Inanition on Responsiveness of the Mammary Gland to Estrogen.** J. J. TRENTIN AND C. W. TURNER, Dept. Dairy Husbandry, University of Missouri. *Endocrinology*, 29: 984. 1941.

Male albino mice were selected to fall within a 15 to 20 gm. weight range at the start of the experiment. The normal daily food consumption per mouse was determined by the daily weight difference of a grain ration provided in a deep container that prevented wastage. This was slightly in excess of 3 gm. daily as determined by individual feeding and collective feeding in groups of 10. The amount of estradiol benzoate required to give unit response at the measured feed levels of 3.0, 2.5, 2.0 and 1.5 gm. per mouse per day was determined and expressed in terms of the amount required to give unit response on unlimited food. As the food intake level decreased the amount of estradiol benzoate required to produce a minimum duct growth response of the mammary glands was considerably and proportionately increased. R.P.R.

- 285. Effect of Stilbestrol on Lactogenic Content of Pituitary and Mammary Glands of Female Rats.** A. A. LEWIS AND C. W. TURNER, Dept. of Dairy Husbandry, University of Missouri. *Soc. Expt. Biol. and Med., Proc.*, 48: 439. 1941.

Stilbestrol treatment of multiparous spayed rats for a period of 10 days caused serous or milk secretion in the partially developed lobule-alveolar mammary glands. The lactogen content of the pituitaries was increased as much as 210% and this was brought about by an increase in pituitary size and an increase in hormone concentration. R.P.R.

## MISCELLANEOUS

- 286. Selection and Operation of Mechanical Refrigeration.** L. C. THOMSEN, University of Wisconsin, Madison. *Natl. Butter and Cheese Jour.*, 33, No. 1: 14. 1942.

The author explains some of the technical aspects of mechanical refrigeration. A formula is given for calculating the rated capacity of an ammonia system. Abbreviated tables showing properties of common refrigerants are given. Dairy plants have a choice of five major systems of refrigeration: the direct expansion system which is used for room cooling, freezing ice



cream, and cooling milk and cream; the brine system, in which brine transmits the refrigeration; the "sweet water" system in which chilled water is used instead of brine; the sweet-water ice storage system which stores refrigeration and transmits it by chilled water; and the use of such substances as propylene glycol to transmit refrigeration. Advantages and disadvantages of each system are given.

The trend toward elimination of gauges is undesirable and decreases possible efficiency; examples are shown with calculations to prove this point. Tables show the proper refrigerant temperatures to maintain desired room temperatures.

W.V.P.

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## ABSTRACTS OF LITERATURE

### ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

- 287. Carotene in Calf Nutrition.** H. A. KEENER, S. I. BECHDEL, N. B. GUERRANT, AND W. T. S. THORP, Pa. State Col., State College, Pa.

Dairy calves of the Holstein and Guernsey breeds were raised to eight months of age on restricted levels of carotene intake. It was found that while the minimum carotene intake which would prevent vitamin A deficiency symptoms during warm weather was about 12 micrograms per pound of body weight per day, the requirement appeared to be about double this amount during cold weather. This observation was further substantiated by a relationship which existed between the environmental temperature and the vitamin A content of the blood. Histological changes were observed in the testicle, kidney, liver and intestines of calves which otherwise would have been considered as normal by the average dairyman. Calves receiving as much as 27 micrograms of carotene per pound of body weight per day were found to exhibit symptoms characteristic of vitamin A deficiency.

- 288. The Use of Ultraviolet Rays in the Cheese Factory and Storage Room.** F. R. SMITH, Univ. Calif., Davis, Calif.

Attempts to control air-borne mold spores by means of 15-watt hot-cathode mercury-vapor lamps have not been effective. Direct exposure of cheese to the ultraviolet rays before and after paraffining failed to prevent mold growth. Data are given on the extent of penetration of ultraviolet rays through some common cheese-coating materials.

- 289. Blind Halves in a Goat's Udder.** C. W. TURNER AND E. R. BEROUSEK, Department of Dairy Husbandry, Univ. Missouri, Columbia.

A case of a purebred Saanen goat is reported which failed to give milk after parturition. Upon anatomical study of the udder it was observed that milk was being secreted in the upper parts of the mammary gland but that the removal of the milk was prevented by the constriction (or blind ending) of the primary milk ducts leading into the cistern of the gland. Reasons are advanced for believing that this condition is due to an inherited or developmental defect in the glands rather than to the development of connective tissue overgrowth resulting from mastitic infection.

- 290. Factors Affecting the Passage of Liquids into the Rumen of the Dairy Calf. II. Elevation of the Head as Milk Is Consumed.**

GEORGE H. WISE, G. W. ANDERSON, AND P. G. MILLER, S. C. Agr.  
Expt. Sta., Clemson, S. C.

The relation of the elevation of the head of the calf to the passage of ingested milk through the esophageal groove into the rumenoreticular cavity has been investigated anatomically and physiologically. The morphology and anatomy of the esophagus and the esophageal groove suggested that the opening and the closing of the lips of the groove are not regulated by mechanical positions and manipulations of the head and neck.

This was substantiated by observations of the physiology of the esophageal groove. Calves were fed from open pails and from nipple pails at floor level and at an elevation corresponding to the height of the withers. The position of the head at these two levels was not a significant factor affecting the frequency of spillage into the two fore compartments of the stomach, but the type of feeder played a very significant rôle. Irrespective of the feeder level, milk rarely entered the rumenoreticular cavity when consumed via nipple but frequently entered when drunk from an open pail.

Application of an electrical stimulus to a vagus nerve of anesthetized calves indicated that the vagi are paths through which certain stimuli may be transmitted to the esophageal groove and the rumen. The transmission and the resulting reaction of the groove were not affected by the position of the head and neck.

## 291. Studies on the Chemical Composition of the Blood of Dairy Cattle.

III. The Normal Concentration of Inorganic Phosphorus in the Whole Blood of Dairy Cattle and Factors Affecting It. A. H. VANLANDINGHAM, H. O. HENDERSON, AND G. A. BOWLING, W. Va. Agr. Expt. Sta., Morgantown, W. Va.

Inorganic phosphorus was determined in more than 600 composite samples of whole blood taken from 59 heifers and cows in normal states of health and nutrition.

When growing dairy heifers were fed rations composed of good alfalfa or timothy hay and a concentrate mixture supplying liberal quantities of phosphorus, and such amounts of Vitamin D as occurred normally in the ration, age was the most important factor effecting the concentration of inorganic phosphorus in the blood.

The inorganic phosphorus in the blood of growing heifers increased with age up to about the seventh or eighth month after which there was a gradual decline as the animals grew older. The inorganic phosphorus in the blood of lactating cows decreased slightly up to about the third or fourth lactation.

Pregnancy caused a decrease in the inorganic phosphorus of the blood particularly during the last six to eight weeks preceding parturition.

There was no indication that the season of the year the heifers were born,

or the fact that they were confined to the barn continuously or permitted to run outside in favorable weather, had any effect upon the inorganic phosphorus content of the blood.

There was a strong tendency for cows beyond the first lactation to show a lower concentration of inorganic phosphorus in the blood during the winter and early spring months than during the summer and early fall.

**292. Phospholipids in Dairy Products. I. Determination of Choline in Dairy Products.** J. C. CRANE AND B. E. HORRALL, Department of Dairy Husbandry, Purdue University Agr. Expt. Sta., Lafayette, Indiana.

A number of experiments were made to determine the consistency of results obtained when extracted butterfat was subjected to relatively mild acid hydrolysis and the choline determined in the neutral or slightly acid hydrolysate by a method which was modified slightly from that of Roman. The values obtained were consistent under the proper conditions, although the choline periodide is unstable at temperatures much above the freezing point. It was further found that in agreement with other work, the factor which was recommended by Roman does not account for all the choline present in aqueous solutions. A corrected factor was used.

Tests made with hydrolysis procedure showed that aqueous normal hydrochloric acid was suitable as a hydrolyzing agent for the splitting of the choline from the lecithin in the extracted fat. A three to five gram sample, or more, was used.

Choline, which was added as freshly prepared egg phospholipids to butter oil, which was low in phospholipids, was recovered by the procedure used. The recovery of choline added as the chloride to butter oil, and to hydrolyzates from extracted milk fat was also carried out.

## BOOK REVIEW

**293. Dairy Engineering.** ARTHUR W. FARRALL, Senior Research Engineer, The Creamery Package Manufacturing Co., Chicago, Ill. John Wiley & Sons, Inc., New York. \$4.00.

Here is a book for which the dairy student has been looking for many years. It is written by one who has the point of view of the dairy engineering and dairy products instructor as well as the commercial operator. Mr. Farrall was formerly Assistant Professor of Dairy Engineering at the University of California and is now Senior Research Engineer for the Creamery Package Manufacturing Co.

Twenty-one chapters and an extensive and valuable appendix and many clear-cut illustrations complete the 405 pages of the book which should

serve as a splendid textbook for dairy manufacturing students of agricultural colleges. The first chapter of the book deals with such physical and chemical properties of milk as are of importance to the dairy engineer. Principles of mechanics, definitions, etc., make up the next chapter. In the following chapters, Power Transmission, Electrical Power and Equipment, Hydraulics and Pumping, Heat Measurements, etc., are covered in detail.

Steam and its use in the dairy, Boilers, etc., are amply covered, and a very complete chapter on Refrigeration follows. This chapter covers principles of refrigeration and various important aspects of dairy refrigeration are well handled.

Chapters include: Insulation, Heaters, Coolers and Heat exchange equipment, Ice Cream freezing equipment, including the various types of freezers, and the operation and factors affecting the same; Homogenization and Pasteurizing Equipment, Evaporating and Drying Equipment, Can Washing and Sterilizing Equipment. Also Bottle Washers, and Fillers, Cream and Butter Handling Equipment, Cheese and Casein Plant Equipment are covered. Equipment maintenance, Pipe Fittings, Fitting and Soldering, Dairy Plant Design, Layout and Utilization are covered in detail. A series of questions follow each chapter.

In the appendix of thirty pages is found a wealth of information useful to the engineer, production manager and student. C.D.D.

## BACTERIOLOGY

- 294. Studies on Lactobacilli. III. Relationship of Immunological Specificity and Fermentative Capacity.** R. W. HARRISON, Walter G. Zoller Memorial Dental Clinics and Dept. Bact. and Parasit., Univ. Chicago. *Jour. Infect. Dis.*, 70, No. 1: 69-76. Jan.-Feb., 1942.

Three hundred and three oral strains of lactobacilli were divided into two groups on the basis of their fermentative ability. Group A produced acid coagulation of milk and fermented sorbitol and mannitol with the production of acid. Group B failed to produce acid from sorbitol and mannitol, but produced acid without coagulation in milk.

Carbohydrate extracts from 75% of the Group A strains, when tested with Group A antiserum, precipitated type specifically. The group B strains, however, behaved as immunologically heterogeneous strains.

J.F.C.

- 295. Studies in Lactobacilli. IV. Changes in Immunological Specificity Associated with Changes in Fermentation Reactions.** R. W. HARRISON, Walter G. Zoller Memorial Dental Clinics and Dept. Bact. and Parasit., Univ. Chicago. *Jour. Infect. Dis.*, 70, No. 1: 77-87. Jan.-Feb., 1942.

Changes in fermentative ability occurred with 26 oral strains of lactobacilli that were being carried on laboratory media. Carbohydrate extracts prepared from these strains after the fermentative changes occurred failed to precipitate with the specific antisera prepared for the strains before the changes occurred. Antisera prepared after the changes precipitated the new carbohydrate extracts, but failed to precipitate the carbohydrate extracts from the corresponding strains before the fermentative changes occurred. Similar but less clear-cut changes in immunological specificity occurred with 7 strains that did not show changes in fermentation reactions.

J.F.C.

- 296. Reclamation of Agar.** HILDA G. MACMORINE, Connaught Labs., Univ. Toronto, Canada. *Pub. Health Jour.*, 33, 1: 39. 1942.

A method is described by which used agar can be freed of extraneous matter and reclaimed. About 75% of the agar used is recovered and when re-used in media gives a hardness only slightly inferior to fresh agar.

O.R.I.

- 297. An Inexpensive Bacteriological Incubator.** C. S. BRYAN AND L. F. JENNINGS. *Vet. Med.*, 36, 11: 567-568. 1941. Abs. from *Mich. Agr. Expt. Sta. Quart. Bul.*, 24, 3. 1942.

Plans are presented for the construction of an efficient, inexpensive bacteriological incubator. The thermostatic control permits the regulation of temperature within the incubator from any point slightly above room temperature to 37° C. An inside temperature of 37° C,  $\pm$  2°, can be maintained under room temperature conditions varying from 1° to 39° C. This incubator is particularly well adapted to the incubation of milk samples for the microscopic test for streptococcal mastitis, but it can be used for other purposes also.

P.H.T.

## BREEDING

- 298. Studies of the Metabolism of Bovine Epididymal Spermatozoa.** GERTRUDE HENLE AND CHARLES A. ZITTLE, Dept. Bact., School of Med., Univ. Pa., Philadelphia. *Amer. Jour. Physiol.*, 136, No. 1: 70. Mar., 1942.

"Variations in the oxygen uptake of bovine epididymal spermatozoa, suspended in Ringer solution, were shown to be due (a) to differences in the individual samples; (b) to the concentration of spermatozoa, optimal respiration taking place in suspensions containing 400 to 800 million cells per ml.; (c) to the pH of the suspensions, highest oxygen consumption occurring at pH 7.5 to 8.0; (d) to possible differences in the degree of maturity of the cells.



"Oxygen consumption of bovine epididymal spermatozoa varied over a wide range. In analyzing this phenomenon it was observed that spermatozoa derived from individual epididymides showed different oxygen needs. Spermatozoa undergo a process of maturation while passing through the epididymis. On frequent emission the cells become more and more immature, while on the other hand spermatozoa stored for a long period in the epididymis lose gradually their fertilizing capacity. These physiological changes may account for some of the individual differences found in this study in that spermatozoa of varying degrees of maturity may have different metabolic requirements. Although the various developmental stages could not be tested, results obtained with seminal and epididymal spermatozoa showed that the oxygen uptake was so much lower in the former than there is no doubt that respiration is less in seminal cells." D.E.

**299. Some Endocrine Relationships in Nutritional Reproductive Failure.**

H. R. GUILBERT, Univ. Calif. Jour. Anim. Sci., 1, No. 1: 3. Feb., 1942.

This is a review of the effect on reproduction, and some possible reactions on endocrine activity, of inanition; of deficiencies of protein and fatty acids; of vitamins A, E, and B (complex); and of the minerals Ca, P, I, and Mn. The author recommends that animal husbandmen give more attention to recording reproductive failures occurring within the borders of their respective states. G.C.W.

## BUTTER

**300. The Surface and Center of Storage Butter.** W. MOHR AND E. SCHRIMPL. *Molk. Ztg. (Hildesheim)*, 53: 1212-1214. 1939.

Water- and air-impermeable paper preserved the surface of storage butter. Surface samples always scored lower in flavor than center samples. There was no correlation of this with chemical differences.

Butter stored for 180 days at  $-4^{\circ}$  F. kept well later at  $60^{\circ}$  F. for 2 weeks after being thawed at the same temperature. J.C.M.

**301. Change Aroma of Butter During Storage.** W. MOHR, E. SCHRIMPL, AND A. ARBES. *Molk. Ztg. (Hildesheim)*, 53: 1501-1503. 1939.

The authors report small quantities of biacetyl in sweet cream butter when churned and after storage. Sour cream butter when made has a significant in flavor percentage of biacetyl, which may remain constant or decrease upon storage. Iron and copper are associated with off-flavors in butter. J.C.M.

- 302. Butter Quality Forecasts.** F. X. MAIER AND F. KIERMEIER. *Molk. Ztg. (Hildesheim)*, 53: 856-861. 1939.

Butter held at 35° C. was scored daily for three months. The yeast and bacteria count after 2 weeks was an index to the quality production. The aldehyde test was used to indicate the yeast count.

The authors concluded that yeasts and bacteria directly and indirectly affected the quality change to a lower grade in 32 and 27%, respectively, of the cases. Metals and oxidation reduced grades in 22% of the samples. The remaining 19% of the samples remained unchanged; and the count after 2 weeks plus the aldehyde determination indicated that in these cases there should be no reduction in quality. J.C.M.

- 303. Studies on Surface Taint Butter. I. Odor Production by *Pseudomonas putrefaciens*.** H. WOLOCHOW, H. R. THORNTON, Univ. of Alberta, AND E. G. HOOD, Dominion Dept. Agr., Ottawa. *Sci. Agr.*, 22, 5: 277. 1942.

*Ps. putrefaciens* inoculated into sterile skim milk (10% spray powder in water) produced a typical "sweaty feet" odor. Butter produced from similarly treated cream showed surface taint defect only when subjected to certain conditions. The evidence suggests that "a precursor is formed in minute amounts in the milk serum under the influence of high temperatures. This precursor appears to follow the casein fraction, . . . *Ps. putrefaciens* appears to act on this precursor to produce the immediate precursor which may then undergo a chemical change, presumably involving oxidation, to form the 'sweaty feet' and surface taint substance."

Neutral or slightly acid conditions, and high heat treatment were necessary for the "sweaty feet" odor to develop in the skim milk or S. T. to develop in the butter. In alkaline skim milk, the odor was putrid. Raw cream and cream pasteurized by the vacreation process did not result in S. T. butter. S. T. production was partially or completely inhibited by the addition of Avenex No. 3, hydroquinone and diacetyl, and Cu changed the odor to one resembling brown sugar. Freshly cut cubes of S. T. butter did not develop the defect when held in an atmosphere freed of O<sub>2</sub>.

Heavier salting and more complete working operated to prevent or delay the appearance of the defect. O.R.I.

- 304. Studies on Surface Taint Butter. II. An Odorous Compound in Skim milk Cultures of *Pseudomonas putrefaciens*.** W. L. DUNKLEY, G. HUNTER, H. R. THORNTON, Univ. of Alberta, AND E. G. HOOD, Dominion Dept. Agr., Ottawa. *Sci. Agr.*, 22, 6: 347. 1942.

Cultures of *Ps. putrefaciens* and *B. subtilis* were grown separately in 10% reconstituted, spray-dried skim milk for two weeks. *Ps. putrefaciens*

gave a "sweaty feet" odor. Steam distillates from both cultures were obtained, concentrated and subjected to Duclaux analyses for volatile fatty acids. Steam distillate residues obtained from both cultures contained formic, acetic, butyric and isovaleric acids. Throughout the analyses the "sweaty feet" odor followed the isovaleric acid fraction. It is concluded that the substance causing "sweaty feet" odor in skimmilk cultures of *Ps. putrefaciens* is closely related chemically to isovaleric acid. O.R.I.

## CHEESE

- 305. Cheese Factory Sanitation.** P. RITTER. Schweiz. Milch Ztg., 65, 26: 135-137. 1939.

The author recommends the reductase test for the receiving platform. Mastitis tests with indicator paper are recommended for regular barn inspections.

The author also favors typing the cheese vat milk for each bath. The pH and propionic acid bacteria should be determined in a cheese sample 24 hours old.

Details for following curd strength, whey acidity and like matters are described. J.C.M.

- 306. Brine for Cheese Making.** G. SCHWARZ AND B. BEINERT. Molk. Ztg. (Hildesheim), 54: 77-78. 1940.

The authors report that brine baths for salting cheese by the immersion method should have a salt content of 18 to 22%. Likewise the acidity should range from .34 to .40 determined with a 9 gram sample of brine following the common test for "acidity" in milk. Weakly acid, neutral or slightly alkali brine solutions are objectionable because of the flora which they favor. J.C.M.

- 307. Studies on Film-Forming Yeasts. II. Film-Forming Yeasts in Rennet Brine.** V. E. GRAHAM AND E. G. HASTINGS, Univ. Saskatchewan and Univ. Wis. Canad. Jour. Res., 20, 2, Sec. C: 63. 1942.

In the commercial production of rennet extract, calves' stomachs are soaked in brine tanks held at low temperature. Unless special precautions are taken, a heavy scum forms on these tanks. Salt-tolerant yeasts of the genus *Debaryomyces* which grow well at low temperatures are chiefly responsible for this scum from which *D. tyrocola*, originally isolated from cheese, and *D. Guilliermondi*, originally isolated from sausages, were isolated. Attempts to isolate these species from the contents of a calf's stomach,

salted calves' stomachs, dried calves' stomachs and soil were unsuccessful. These species did not grow in a medium containing 20% sodium chloride, nor in one in which the pH had been lowered to 2.0. O.R.I.

## CHEMISTRY

- 308. Determination of Total and Inorganic Bromide in Foods Fumigated with Methyl Bromide.** S. A. SHRADER, A. W. BESHGETOOR AND V. A. STENGER, Dow Chemical Co., Midland, Mich. *Jour. Ind. Eng. Chem., Analyt. Ed.*, 14, No. 1: 1. 1942.

A method is proposed whereby methyl bromide is removed under conditions which lead to minimum decomposition and it is determined as the difference between total bromide and inorganic bromide. Application to several different types of food products including American cheese indicates that no methyl bromide remains as such for more than four days after fumigation. B.H.W.

- 309. Colorimetric Determination of Phosphorus in Biological Materials.** RUTH ADELE KOENIG AND C. R. JOHNSON, Texas Univ., Austin, Texas. *Jour. Ind. Eng. Chem., Analyt. Ed.*, 14, No. 2: 155. 1942.

Misson's spectrophotometric method for the estimation of phosphorus has been improved for use in biological materials by increasing its range, sensitivity and precision. A description of the improved method, its applications and a summary of typical results in various materials including milk powder are presented. B.H.W.

- 310. Determination of Citric Acid in Pure Solutions and in Milk by the Pentabromoacetone Method.** EDGAR F. DEYSHER AND GEORGE E. HOLM, U. S. Dept. Agr., Bur. Dairy Ind., Washington, D. C. *Jour. Ind. Eng. Chem., Analyt. Ed.*, 14, No. 1: 4. 1942.

"The determination of pentabromoacetone formed by the action of potassium permanganate and bromine upon citric acid under controlled conditions has been used as a basis for the quantitative determination of citric acid by various authors. The recovery of citric acid by the methods employed has usually been less than the theoretical value, especially where sugars have been present." Losses may be incurred through incomplete conversion of citric acid to pentabromoacetone, by volatility of the precipitate or by its solubility in the reaction mixture and wash water. A study of losses was made and suggestions are advanced for aiding in a more complete recovery of citric acid as pentabromoacetone. "The modified method applied to aliquote of solutions containing from 0.10 to 0.30 gram of citric acid gave results that were consistently within  $\pm 0.50\%$  of the theoretical values.

When the quantity of citric acid was less than 0.10 gram the percentage recoveries were slightly less than the value given and the results were not so consistent as those obtained with the use of larger amounts. In the case of milk the quantity of serum that can be conveniently used in each determination is such that the approximate amount of citric acid present is less than 0.10 gram. The results obtained upon decitrated milks to which citric acid in these amounts had been added were usually within  $\pm 1.00\%$  of the theoretical values, though the differences between duplicate determinations were usually greater than those in which larger amounts were present."

B.H.W.

### CONCENTRATED AND DRY MILK; BY-PRODUCTS

311. **Production of Textile Casein.** F. RICHTER. *Milk. Ztg.* (Hildesheim), 53: 1163-1168. 1939.

Sulphuric acid-precipitated casein is desired for textile use. The whey obtained from this method is rendered suitable for stock feed by adding  $1\frac{1}{2}$  pounds of an equal mixture of calcium carbonate and calcium hydroxide per 1000 pounds of whey.

J.C.M.

312. **Production and Consumption of Manufactured Dairy Products.** E. E. VIAL, U. S. Dept. Agr., Tech. Bul. 722.

This publication discusses briefly the historical aspects of production trends of the different manufactured products starting with the year 1849. The per capita consumption of all manufactured dairy products (milk equivalent) rose from 305 pounds in 1870 to 421 pounds in the period 1930-1937. The upward trend prior to 1900 was due primarily to the increasing per capita consumption of butter, and since then to the upward trend in the consumption of cheese, concentrated milks and ice cream. Domestic consumption has increased somewhat more rapidly than domestic production.

In the past 85 years the long-term tendency has been for butterfat prices to rise in relation to prices of feed grains, which has been an important factor in causing farmers to expand dairying in relation to some other types of agricultural production.

P.H.T.

### DISEASE

313. **Persistence of Antibodies One Year After Active Immunization of Human Beings with a Mixed Heat-Killed Vaccine of *B. typhosus*, *Br. abortus* and *Br. melitensis*.** JOHN A. KOLMER, AMADEO BONDI, JR., AND ANNA M. RULE, Res. Inst. Cutan. Med. and Dept. Bact. and Immunol., Temple Univ. School of Med., Philadelphia. *Jour. Infect. Dis.*, 70, No. 1: 54-57. Jan.-Feb., 1942.

Tests were made with the serums of 29 normal human adults that had been immunized one year previously with a mixed heat-killed vaccine of *Bacillus typhosus*, *Brucella abortus* and *Brucella melitensis*. The tests made were for H and O typhoid agglutinins, *Br. abortus* agglutinins, *Br. abortus* opsonins, and serum protection tests in mice with all three organisms.

With few exceptions there was a decrease in serum potency of all of the immunized individuals as compared to tests that had been made two weeks following immunization. However, with all tests a high percentage of the serums (56 to 100%) showed potency levels still higher than the pre-immunization levels. J.F.C.

**314. Calf Scours.** C. F. CLARK, East Lansing, Mich. Mich. Agr. Expt. Sta. Quart. Bul., 24, No. 2: 99-100.

Specific remedies for the treatment of both white and dietary scours are given. Emphasis is placed upon prevention, however. The preventive measures recommended for both types of the disease are as follows: 1. Provide a clean, dry, well-bedded maternity stall in which the cow may calve. It is wise to clean and disinfect the maternity stall after each cow has calved there. It is presumed that the cow has been properly fed so that there will be adequate vitamin A in the milk on freshening. 2. Disinfect the navel of the calf with tincture of iodine as soon after birth as practicable. 3. Make certain that the calf receives colostrum milk as early as possible, by assisting it to nurse if necessary. Check to be certain that some milk is obtained from each quarter of the udder. 4. Do not allow the calf to consume an overload of colostrum. The present-day high-producing dairy cow produces more milk than even the most vigorous calf can successfully consume. If the calf is to be pail-fed it may learn to drink more readily if removed from the cow soon after active bowel function is established. 5. Continue the feeding of dam's milk for a week after birth. 6. Begin pail feeding by giving milk amounting to 6-8 per cent of the body weight per day. Feed at regular hours and intervals, in clean pails, at a temperature of 98-100° F. Weak calves may do better if fed three times daily. 7. Keep the calf pens well bedded and dry. Elevated floors, of heavy, expanded metal, are excellent. Single pens are to be preferred. If several calves must be kept together, it may be well to tie them after feeding milk so as to discourage sucking. 8. If scouring or other disease appears in calves, consult the best qualified veterinarian possible to employ. P.H.T.

## FEEDS AND FEEDING

**315. Management Practices and Returns on White Clover Pastures.**

E. VANDER MEULEN, G. MCINTYRE, AND C. M. HARRISON, East Lansing, Mich. Mich. Agr. Expt. Sta. Quart. Bul., 24, 3: 233-238. 1942.

The white clover has largely disappeared from pasture land in Michigan due to mismanagement and lowered soil fertility. Areas in which white clover can be re-established are generally confined to the cool, moist regions located in the northeastern portion of the lower peninsula, and in the upper peninsula. The plant is most productive on the heavier soils which are low-lying or have characteristics of poor drainage.

Top-dressings of phosphatic fertilizers coupled with a close grazing by livestock are essential to the maintenance of the plant. P.H.T.

**316. Legume Silage vs. Corn Silage vs. Legume Hay for Fattening Heifer Calves.** G. A. BRANAMAN AND G. K. DAVIS, East Lansing, Mich. Mich. Agr. Expt. Sta. Quart. Bul., 24, 3: 227-232. 1942.

The object of this experiment was twofold: (1) to compare alfalfa hay and alfalfa silage when equal amounts of corn are fed, and (2) to determine the amount of corn required with alfalfa hay or alfalfa silage to equal well-eared corn silage. Texas-bred Hereford heifer calves of "good" grade were used in this test. The alfalfa hay and silage were made from first-cutting alfalfa containing a heavy timothy mixture. For the ensilage 40 pounds of beet molasses was mixed with each ton of alfalfa. The cattle ate the silage more readily than they did the hay, and their gains in weight were more consistent. The grass silage must be supplemented with grain in order to make it comparable to corn silage. Under the conditions of the experiment one pound or more of corn was added for each 100 pounds of body weight. Calves fed corn silage gave a higher return per head above feed cost and a higher value per ton of silage than those fed alfalfa silage. The hay-fed cattle graded lowest and the alfalfa silage-fed cattle highest in carcass grade. The corn silage-fed cattle graded highest in color of fat, while the alfalfa silage-fed cattle graded lowest. P.H.T.

**317. Carotene in Feed Grasses.** JOHN ALLARDYCE AND DOUGLAS MILSOM, Univ. Brit. Columbia, Vancouver. Canad. Jour. Res., 20, 2, Sec. C: 85. 1942.

Cuttings of feed grasses less than 7 inches high were found to vary considerably on carotene content. Factors that were found to contribute to these variations were: (1) the amount of sunshine and rain prior to cutting, (2) the height of the cutting, and (3) the manner of storage. Higher carotene content was found when the cuttings were taken following periods of increased daily sunshine. Cuttings less than 7 inches high contained larger amounts of carotene than did 12 inch cuttings. Storage of the ground, dehydrated feed grasses at 35° F. for three months reduced the carotene content by 7.5%. O.R.I.

- 318. Seasonal Changes in the Lignin and Cellulose Content of Some Montana Grasses.** A. R. PATTEN AND LEONARD GIESEKER, Mont. Agr. Exp. Sta., Bozeman. Jour. Anim. Sci., 1, No. 1: 22. Feb., 1942.

Lignin and cellulose were determined in 5 species of grass, at 5 stages, in 2 localities. The species included 3 wheat grasses and 2 brome grasses, and the stages ranged from the first evidence of flower stalks to shattered seed heads. In the first stage the lignin content was slightly over 5 per cent and the cellulose content was slightly over 20 per cent in all cases. In the last stage the lignin content had increased about threefold and the cellulose less than twofold. In the more arid area the grasses reached the maximum lignin content earlier. As lignin is practically indigestible this gives further evidence that more attention should be given this substance in forage analyses. G.C.W.

- 319. The Metabolism of Calcium and Phosphorus as Influenced by Various Activated Sterols.** E. W. MCCHESENEY AND FREDERICK MESSER, Res. Labs., Winthrop Chemical Co., Rensselaer, N. Y. Amer. Jour. Physiol. 135, No. 3: 577-586. Feb., 1942.

The effects of single massive doses of vitamin D<sub>2</sub>, D<sub>3</sub> and of Ertron (a form of irradiated ergosterol) and A.T. 10 (dihydrotachysterol) on the calcium and phosphorus metabolism of dogs have been compared. As to serum calcium, vitamin D<sub>2</sub> and Ertron are essentially the same in their effects. Vitamin D<sub>3</sub> is characterized by the long persistence of a rather moderate degree of hypercalcemia. "A.T. 10 causes a very rapid rise of serum calcium followed by a comparatively rapid fall. All the products cause a rise in serum phosphorus (Ertron not studied). All of the products decrease fecal and increase urinary output of calcium. They also increase fecal output of P and either increase urinary output or maintain it at a constant level in spite of decreased intake. All of the products except vitamin D<sub>3</sub> improved calcium balances; Ertron improved the phosphorus balance slightly." D.E.

## FOOD VALUE OF DAIRY PRODUCTS

- 320. Fats and Oils.** ELIZABETH F. WHITEMAN AND FLORENCE B. KING, U. S. Dept. of Agr., Leaflet 204. 1940.

This article, prepared primarily for the housewife, contains useful information regarding the energy value, vitamin value, and digestibility of the various fats and oils commonly used in the household (butter, oleomargarine, lard, hydrogenated fats and compounds, and salad oils). Explanation is also given of the use and care of these various oils and fats. P.H.T.



- 321. Is There Need for the Fortification of Milk?** E. V. McCOLLUM, Johns Hopkins Univ., School of Hygiene and Public Health, Baltimore, Md. *Amer. Jour. Pub. Health*, 32, No. 1: 80-84. 1941.

The conclusion is made that it is unwise to permit fortification of milk other than with Vitamin D. It is unnecessary in the interest of the consumer and undesirable from the standpoint of the distributor. There is no advantage in making any single food a complete food for all nutritional purposes. We will fare best by taking a variety of foods, each of which provides something which is needed. M.W.Y.

## ICE CREAM

- 322. Sweeteners Used in Ice Cream.** B. E. HORRALL, Purdue Univ. Agr. Expt. Sta., Lafayette, Ind. *Ice Cream Field*, 34, 2: 28. 1942.

The author gives in concise form the generally accepted recommendations regarding the use of sweetening agents that can replace part of the sucrose in ice cream. He refers to the work of various investigators which shows that corn sugar, invert sugar, honey, certain corn syrups and dried corn syrups can be used up to 25% of the total sugar without sacrifice of body and texture in the finished ice cream. W.C.C.

## MILK

- 323. Milk for the Family.** ROWENA S. CARPENTER, U. S. Dept. Agr., *Farmers Bulletin* 1705. 1940. (This bulletin supersedes *Farmers Bulletin* 1359, issued 1933.)

This publication of 29 pages is a popular treatment of milk from the standpoint of its use in the home. An explanation of the products manufactured from milk is also given. The nutritive value of milk is discussed, the pasteurization process is explained, and the different commercial grades of milk are defined. Directions for the proper care of milk in the home are given and a brief discussion of the use of milk in cooking is presented. P.H.T.

- 324. Sour Cream—How to Prepare and Use It in the Home.** U. S. Dept. Agr., Leaflet 213. 1941.

Methods for making sour cream suitable for home operations are presented. Twenty different recipes for the use of sour cream are given. This material would be useful for encouraging milk customers to make more extensive use of sour cream as sold by milk plants. P.H.T.

- 325. E. O. D. Delivery—The Ruination of the Small Dealer.** W. E. DONOHUE, David Donohue & Sons, Holyoke, Mass. *Milk Dealer*, 31, 5: 56-58. Feb., 1942.

The author points out that the every-other-day delivery plan suggested to relieve the tire situation is the brain child of the milk trusts; that small dealers and milk plant owners haven't a chance of surviving against such a scheme; that they would lose a good part of their volume and patronage to the stores, as many of the multiple quart customers will not sacrifice space in their refrigerators to hold two days' milk supply. This drop in volume would put the small dealers out of business to the benefit of the large dealers with wholesale store routes. It is suggested that the six-day delivery plan should be adopted and in cases where it is possible, to have two dealers combine their routes, using one truck one day and the other the following day.

C.J.B.

- 326. "Do's and Don'ts" in Handling Cream.** L. R. GLAZIER, Dairy Engineer, The Pfaudler Co. *Milk Dealer*, 31, No. 5: 94. Feb., 1942.

A convenient tabulation of the causes and methods of preventing the following cream defects is given: 1. Cream plug. 2. Feathering. 3. Foaming. 4. High acidity. 5. Lipase (enzyme) activation. 6. Off-flavors. 7. Oiling off. 8. Poor whipping ability. 9. Poor viscosity. 10. Serum separation.

C.J.B.

- 327. A Method of Determining Fat in Homogenized Milk.** J. C. MARQUARDT, N. Y. Agr. Expt. Sta., Geneva, N. Y. *Milk Dealer*, 31, No. 5: 24. Feb., 1942.

The author presents a modification of the Babcock fat test which gives clear fat columns with homogenized milk. The modification consisted in adding the acid in three equal charges, shaking well after each addition. Following the first centrifuging the samples were removed and thoroughly shaken. This was repeated after half of the first addition of water was made, and again after the completion of the water addition prior to the second centrifuging. After the second run the samples were again shaken before the final addition of water.

C.J.B.

- 328. The Place of Ice Milk in the Dairy Industry.** HARRY B. BURT, Pres., Malt-A-Plenty, Inc., Tulsa, Okla. *Ice Cream Field*, 34, No. 2: 24, 32, 33. 1942.

The greatest development of ice milk has occurred in California, where the manufacturer and the public have cooperated to regulate and produce a nutritious, healthful product. Ice milk is defined as "a wholesome dairy product, frozen in the same manner as ice cream but with a butterfat content approximating that of whole milk."

Little difficulty with ice milk has been encountered in localities where its production is governed by law or regulation; manufacturers, however, in

localities where regulations do not exist often experience considerable difficulty. It is claimed that low-income groups, unable to purchase ice cream, can afford ice milk. This accounts for its greater success in the southern states.

The author claims that ice milk has a greater digestibility than high-fat ice cream. (Abstractor's note: No specific evidence is given to substantiate this claim.)

It is stated that ice milk is not a substitute for ice cream, but that it should be given recognition and be regulated along with ice cream. It is advocated that those in the industry should strive to gain a Federal Standard for ice milk.

W.C.C.

**329. Metallic Flavored Milk.** H. HERZFELD AND E. VOLLBRECHT. Deut. Molkerei-Ztg., 60: 635-671. 1939.

Copper and iron rods were used to stir pasteurized milk for two minutes to demonstrate metal effects upon flavor. Both procedures gave comparable metallic flavors when the stirring was at 176° F. However, decreasing the time factor proved that copper was far more harmful than iron. The authors failed to associate intensity of the off-flavors with quality of the raw milk.

J.C.M.

**330. Separating Sour Cream Buttermilk.** W. MOHR, A. ARBES, AND M. KELTING. Mol. Ztg. (Hildesheim), 53: 2153-2155. 1939.

It is possible according to the authors to separate sour cream buttermilk in a foamless separator. The separating is best achieved at 60° F. with the adjustments made for a 20% cream. The yield of 20% cream is about 15 pounds per 1000 pounds of sour cream buttermilk. This results from a sour cream buttermilk containing .45% of fat. The yield is greatly increased when the buttermilk contains, as is sometimes the case, more than 1% of fat. The cream obtained is equal in physical and general character to normal cream.

J.C.M.

**331. Studies on the Antioxygenic Effect of Oat Flour Treated Milk Bottles for Milk Exposed to Sunlight.** C. W. ENGLAND AND ARTHUR P. WIEDEMER, Md. Agr. Expt. Sta., College Park, Md. Milk Dealer, 31, No. 3: 33-36. Dec., 1941.

Report of a study to determine the value of oat flour in preventing sunlight-induced oxidized flavors in market milk. The following conclusions are drawn: 1. Increasing the length of time milk is exposed to sunlight increases the intensity of the oxidized flavor. 2. Midday sunlight induces more intense oxidized flavors in milk in glass bottles than morning and afternoon sunlight; while morning sunlight induces more intense oxidized flavors in milk

in paper bottles than midday and afternoon sunlight. 3. The exposure of milk at 70° F. to sunlight results in increased oxidized flavor development compared to milk exposed at 40° F. 4. Paper bottles offer more protection to milk from sunlight oxidation than glass bottles. 5. Paper bottles treated with oat flour when exposed to sunlight for 10 minutes or less afford little protection to the development of sunlight-induced flavors in milk as compared with plain paper bottles. 6. Oat flour treated paper bottles offer some protection to milk against oxidized flavors when the period of exposure to sunlight exceeds 10 minutes. C.J.B.

Editor's comments: The oxidized flavor described should not be confused with the tallowy or oxidized flavor induced by copper contamination. The "sunshine" flavor is in reality an effect of the light rays upon the milk protein.

**332. This Milk Distribution Problem.** EDWARD THOM, Assoc. Ed., *The Milk Dealer*. *Milk Dealer*, 31, No. 3: 22-23, 48-50. Dec., 1941.

The milk distribution problem is discussed mainly from the standpoint of six-day delivery. The advantages of six-day delivery are summed up as follows: 1. Puts the regular route man in full control of his route, which results in more uniform and accurate service at all times. 2. Driver salesmen like it because they always know they will have Sundays to themselves. 3. Makes it possible to cut cost of distribution. 4. Makes the job of delivery more desirable and makes it possible to obtain better men more easily. 5. From a public relations standpoint, it is considered more valuable than seven-day delivery. 6. Relieves the management of the greater part of Sunday responsibilities. 7. Customers become accustomed to quantity purchases, which apparently results in the greater consumption of milk. C.J.B.

**333. Factors Affecting the Sale of Milk.** DR. L. D. H. WELD, Dir. Res., McCann-Erickson, Inc. *Milk Dealer*, 31, No. 4: 74-86. Jan., 1942.

Such factors as Package Cost, Price Spread, Increasing Consumption, Vitamin Appeal, Increased Buying Power, Consumer Attitude, etc., are discussed in their relation to the sale of milk. The following promotion suggestions are offered:

1. Stress the importance of adequate daily amounts of milk. Consumers over-estimate the amounts consumed and they must be made definitely conscious of the quantity that they drink and the quantity that they ought to drink.
2. Direct the campaign largely to adults. Adults form two-thirds of the population and only half of them drink milk now.
3. Direct the campaign to both men and women. The deficiency is nearly equal between men

and women. 4. Feature milk drinking by showing how its nutritional value is greater than that of other beverages. 5. Play up the use of milk between meals and at bed-time. 6. Meet the taste factor squarely. When milk is played up as a part of appetizing meals or with other foods, a real appetite appeal for it can be developed. 7. Stress the economy of milk. Show that as compared with other foods milk is cheap. 8. Tie in your general nutrition story with the government program. 9. Use strong emotional appeals to urge action. People have a high regard for the value of milk and this proves that the educational work that you have done has been effective. You will get the market ready and keep it ready through additional effort spent in advertising.  
C.J.B.

**334. Milk Service for Factories.** ANONYMOUS. *Milk Dealer*, 31, No. 4: 30-31, 46-47. Jan., 1942.

The milk service for factories based mainly on the experience at the Thermoid plant in Trenton, New Jersey, where the plan to serve a half-pint of milk daily was inaugurated in April, 1941, is discussed. Since the plan was inaugurated it is estimated that the plant has had: 1. A 30% reduction in accidents, with an improvement in safety. 2. Fewer absences due to illness, indicating a general improvement in employees' health. 3. An increase in production during those hours that were formerly low points in the employees' day. Since practically all workers are on a piece-work basis, their incomes have increased.  
C.J.B.

**335. Accidents Don't Happen.** ROBERT I. GAYLEY, Safety Director, Supplee-Wills-Jones Milk Co., Philadelphia, Pa. *Milk Dealer*, 31, No. 4: 22-23. Jan., 1942.

A description is given of how the Supplee-Wills-Jones Milk Co. is attacking the problem of safe driving from a new angle. Actual driving contests, including such operations as backing, holding a line, blind and sight side-parking, and turning in a restricted area, are conducted. According to the author there are two main problems which any safety director of a large company must face. First he must try to eliminate the causes of accidents before they occur. Second, he must overcome the smugness of the average driver who believes he has nothing to learn about the safe operation of a vehicle.  
C.J.B.

**336. Streamlining Our Milk Delivery System.** ROICE ANDERSON AND LELAND SPENCER, Cornell Univ., Ithaca, N. Y. *Milk Dealer*, 31, No. 5: 22-24, 48-54. Feb., 1942.

A discussion is given of methods to conserve tires and reduce other expenses. The discussion is summarized as follows:

The most promising change in the system of milk deliveries from the point of view of tire conservation and cost saving is the adoption of alternate-day delivery. The indications are that savings of from 20 to 35% can be made in this way. By introducing an appropriate schedule of discounts for volume deliveries to consumers, any distributor can proceed with alternate-day delivery whether or not the plan is adopted by his competitors. Even where the alternate-day system is not adopted, a considerable saving in delivery time, though not in truck mileage, can be accomplished by the use of volume discounts or the use of larger-than-quart-size containers to encourage customers to take their milk on alternate days.

Smaller but considerable savings can be made in other ways:

(1) By discounting or exchanging customers who are expensive to serve, such as customers at a distance from main route, single or scattered stops in apartment houses, and split stops at stores, restaurants, etc. (2) By drastically curtailing call-backs and special deliveries. (3) By changing from early-morning to daylight delivery.

Providing the proper steps are taken to explain the need for such changes, and providing appropriate discounts are offered in recognition of the lesser cost of more limited service, it is more than likely that consumers will respond favorably.

A change from the competitive system of milk delivery to a unified or monopoly system would be much more difficult to accomplish than any of the other changes suggested. The limited experience with milk distribution under public or private monopoly gives little basis for judging the probable results from the standpoint of efficiency. Any plan of unification so far conceived would involve not only a consolidation of delivery service, but the elimination of private brands as well. It seems unlikely that much progress toward unification will be made except as a war measure, by direct order of the national government.

C.J.B.

**337. Ramblings on Vending.** ROY K. QUINLAN, Quinlan & Baker, Providence, R. I. *Milk Dealer*, 31, No. 2: 42-46. Nov., 1941.

The author discusses milk vending from the standpoint of causes for failure. The most frequent causes of failure are: Not enough attention to where the machine is located, incompetent salesmen, and poor servicing.

C.J.B.

**338. Stability of the Fat Emulsion of Homogenized Milk.** F. J. DOAN, D. V. JOSEPHSON, AND JAMES ADAMS, Pa. Agr. Expt. Sta., State College, Pa. *Milk Dealer*, 31, No. 2: 35, 54-60. Nov., 1941.

Report of a study to determine whether or not the requirement for homogenized milk as defined by the U. S. Public Health Service milk ordi-

nance and code is too stringent. This ordinance defines homogenized milk as follows:

"Homogenized milk is milk which has been treated in such a manner as to insure break-up of the fat globules to such an extent that after 48 hours' storage no visible cream separation occurs on the milk and the fat percentage of the top 100 cc. of milk in a quart bottle, or of proportionate volumes in containers of other sizes, does not differ by more than five per cent of itself from the fat percentage of the remaining milk as determined after thorough mixing."

The following conclusions are drawn: 1. Much of the commercial homogenized milk sold fails to meet the requirements for the product stipulated by the U. S. Public Health Service definition. 2. A method of removing the top 100 ml. of homogenized milk from a quart bottle for the purpose of determining whether it conforms to the United States Public Health Service definition is described. 3. By this method analyses of duplicate bottles check very closely, even though it is visually evident that some fat is not removed in the upper 100 ml. from unstable samples of milk. 4. A modification of the Babcock test is described for homogenized milk. This test checks as closely as can reasonably be expected with the Mojonnier method and United States Public Health Service Indices of fat stability calculated from modified Babcock tests and from Mojonnier tests agree very closely, indicating that the Babcock method is satisfactory for the purpose. 5. The fat content of homogenized milk (between 3.5 and 4.5%) has only a very small and inconsistent effect on the stability of the fat emulsion. 6. The rotary homogenizer used in the study is more efficient in stabilizing the fat emulsion of homogenized milk than the piston machines used, when similar pressures are employed. 7. Piston homogenizers, of the types used, must be operated at pressures exceeding 3,000 pounds to consistently produce milk meeting United States Public Health Service requirements. The rotary and centrifugal homogenizers used cannot satisfactorily meet this definition. 8. An extremely close relationship exists between the United States Public Health Service Index and the Farrall Index of efficiency of homogenization. A Farrall Index of approximately 20 corresponds with a United States Public Health Service "per cent difference" of five. 9. The United States Public Health Service definition of homogenized milk is unreasonably stringent.

C.J.B.

**339. The Influence of the Time and Temperature of Homogenization on Certain Properties of Milk.** G. M. TROUT AND M. V. SCHEID, East Lansing, Mich. Mich. Agr. Expt. Sta. Quart. Bul., 24, No. 2: 122-131.

Raw milk was held at 40° F. for 24 hours before homogenizing at 5000 pounds pressure at 40°, 60°, 100°, 120° and 140° F. Complete dispersion

of the fat did not occur until a temperature of 100° F. was reached. Marked increases in acidity resulted when the raw milk was homogenized at 80°, 100° and 120° F. At 60° and 140° the increases were slight and at 40° F. there was no change in acidity.

When milk homogenized raw at 130° F. was immediately pasteurized, no harmful flavor effects were noted. Raw milk vigorously agitated at 40° F. failed to develop an increased acidity or a rancid flavor.

When milk is to be homogenized before pasteurization it must be preheated high enough to inactivate the lipase or the plant facilities must provide for immediate pasteurization after homogenization. P.H.T.

**340. Foaming of Homogenized Milk.** G. M. TROUT AND M. V. SCHEID,  
East Lansing, Mich. Mich. Agr. Expt. Sta. Quart. Bul. 24, No. 2:  
113-115.

Raw milk homogenized at 100° F. and then pasteurized showed less foaming than that homogenized after pasteurizing. Slightly less foaming occurred when the milk was homogenized after pasteurization if the temperature was lowered to 100° F. before homogenization took place. The foaming of homogenized milk can be best controlled by exercising caution in operation such as: 1. Operating the bottles at slow speed. 2. Maintaining a maximum head of milk in the supply tank. 3. Reducing splashing of milk at cooler spreader pipe to minimum. 4. Reducing drop from cooler coils to the cooler reservoir. 5. Having air-tight connections in the lines.

P.H.T.

**341. Overcoming Seepage of Bottled Homogenized Milk.** G. M. TROUT,  
East Lansing, Mich. Mich. Agr. Expt. Sta. Quart. Bul., 24, 3:  
217-227. 1942.

To overcome seepage of bottled homogenized milk due to expansion of the milk it is recommended that the filler valves be adjusted so that 56-mm. bottles will not be filled closer than one-fourth inch from the cap seat. Such may not be the case with the 48-mm. bottle, however, which when filled to the bottom of the lip roll must be kept from warming appreciably if seepage is to be overcome.

Bottles of homogenized milk should be handled in such a manner during distribution as to keep the milk out of contact with the cap as much as possible. The increased capillarity and penetrability of milk due to homogenization coupled with an expansion of the milk may result in seepage if the milk is allowed to contact the closure seat regularly. P.H.T.



## PHYSIOLOGY

- 342. Effect of the Gonadotropic Substance of Pregnant Mare's Serum on the Blood Plasma-Ascorbic Acid of the Bovine.** RALPH E. ERB AND FREDERICK N. ANDREWS, Purdue Univ. *Endocrinology*, 30, No. 2: 258. Feb., 1942.

Two bulls and 4 cows were injected subcutaneously with amounts of gonadotropic hormone (Gonadin) varying from 1000 to 2250 rat units during 8 individual trials. In certain of these experiments crystalline vitamin C was administered subcutaneously in sterile saline solution. The gonadotropic hormone caused a decrease in venous blood plasma-ascorbic acid (42 to 67%) of the two bulls during the first 24-hour period. The hormone treatment caused decreases of 20 to 50% during the first 24-hour period in the 4 cows. The recovery of blood plasma-ascorbic acid to approximately pre-injection levels required 8 to 10 days in the cow and longer in the bull unless extraneous vitamin C was injected. R.P.R.

- 343. The Effect of Thyroidectomy on Lactation in the Albino Rat.** DAVID KARNOFSKY, Stanford Univ. *Endocrinology*, 30, No. 2: 234. Feb., 1942.

Virgin albino rats weighing 200 gm. and about 140 days old were thyroidectomized before conception, during gestation, or immediately after delivery. All litters were reduced to 5 but frequently death of the young further reduced this number. The young were weighed daily and the weights used as an indication of milk production. The results indicated that: (1) the thyroid gland does not appear to be essential for lactation in the rat; (2) thyroidectomy immediately after delivery, during gestation, or before conception causes a decrease in milk production; (3) thyroidectomy before conception or during pregnancy does not inhibit mammary gland development; and (4) thyroid replacement in thyroidectomized rats probably increased milk production. R.P.R.

- 344. Changes in the Fat Percentage and Fat Yield of Dairy Cows with Injections of an Anterior Pituitary Preparation.** J. F. SYKES, W. L. MEULEMAN, AND C. F. HUFFMAN, Mich. State Col. *Endocrinology*, 30, No. 2: 217. Feb., 1942.

Four cows, 3 Holsteins and one Jersey, in declining lactation were used for experimentation. Milk fat percentage was determined on 3-day composite milk samples. A fat metabolism preparation of the anterior pituitary was injected for 5-day periods in 500-mg. amounts. For comparative purposes a prolactin preparation was injected in 500-mg. amounts into the same 4 cows during another 5-day period. The fat metabolism preparation

caused a marked increase in the fat production in 3 of the 4 injected cows and slight increases in milk volume in each cow. The prolactin injections caused similar increases in milk volume but had no detectable effect on milk fat percentage.

R.P.R.

- 345. The Influence of Ascorbic Acid on the Activity of Gonadotropic Hormones.** ALFREDO V. DI CIO AND MARIO SCHTEINGART, Clinic of Dr. Mariano R. Castex. *Endocrinology*, 30, No. 2: 263. Feb., 1942.

The simultaneous injection of 25 rat units of gonadotropic hormone and 50 mg. of ascorbic acid daily for 20 to 30 days into 2- to 4-month-old rats produced a greater increase in development of testis and penis, and of female genitalia than the hormone alone. The testicular weight was almost doubled by the combined treatment as compared with the hormone alone. Ascorbic acid alone produced no increase in the weight of the testes. There appeared to be no effect from injecting the vitamin into adult animals or over a 3-day period.

R.P.R.

- 346. Initiation of Lactation in Nulliparous Heifers by Diethylstilboestrol.** S. J. FOLLEY, H. M. SCOTT WATSON (MRS. C. C. THIEL), AND A. C. BOTTOMLEY, Nat. Inst. for Res. in Dairying, Reading. *Jour. Physiol., Proc.*, 100, No. 3: 7-8. Nov., 1941.

The application of five grams of diethylstilboestrol dipropionate ointment three times weekly to the udders of two nulliparous Dairy Short-horn heifers (18½ and 20 months of age) resulted in a maximum secretion of 170 ml. of fluid daily. "The composition of the secretion varied somewhat at different periods of the experiment but never resembled that of normal milk, since it always contained less casein and more globulin. At times the fat content approached normal but the non-fatty solids were usually deficient."

D.E.

- 347. Copper-Induced Pseudopregnancy in the Adult Estrous Rat.** A. DURRY AND J. T. BRADBURY, U. S. Dept. Agr., Div. Nutr. and Physiol., Bur. Dairy Ind. *Amer. Jour. Physiol.*, 135, No. 3: 587-590. Feb., 1942.

"Intravenous administration of copper solutions induce pseudopregnancy in the adult estrous rat. The minimal effective dose is 0.1 cc. (0.3 mgm. of copper ion) of a 1% copper acetate solution. Approximately 1 cc. of the same solution is the minimal ovulating dose in the estrous rabbit. These data suggest that copper may act through the pituitary. They also suggest that a fundamentally similar neuroendocrine physiology exists in

both spontaneously ovulating rat and the non-spontaneously ovulating rabbit."

The Bureau of Dairy Industry also reported on the gonad-stimulating materials in plants in the Report of Chief of the Bureau of Dairy Industry, 1941, p. 30. These two reports have many points of common interest.

D.E.

### MISCELLANEOUS

- 348. Proper Lubrication—A War Time Defense Job.** EDWARD THOM, Assoc. Ed., *The Milk Dealer*. *Milk Dealer*, 31, No. 5: 31-34, 42-44. Feb., 1942.

A discussion is presented of the necessity for proper lubrication and how it can be best accomplished. A follow-up file is suggested in order to be certain that all moving parts are lubricated at the proper time. The use of the proper lubricant, depending on type and size of machine, speeds, temperatures, and surrounding conditions with respect to moisture, dust, etc., is stressed. The lubrication of bottle washers, refrigerator equipment, conveyors, etc., is then discussed.

C.J.B.

- 349. Adjustable Wage Scale Based on Changing Cost of Living Adopted by Louisville Dealers.** ANONYMOUS. *Milk Dealer*, 31, No. 3: 68-70. Dec., 1941.

A brief description of the automatic wage-adjustment plan used by Louisville dealers-employees is presented. Hourly wage rates under this plan are based on the commodity price index. A table showing the changes in hourly wages with changes in the commodity index is given.

C.J.B.

- 350. Paraffin Wax.** EDGAR F. WRIGHT, Mono Service Co., Newark, N. J. *Milk Dealer*, 31, No. 4: 28-29, 50. Jan., 1942.

A discussion of the use of paraffin wax in the dairy industry and its war uses is given. With a view to helping the O.P.M. and the dairy industry in conserving paraffin wax the following suggestions are offered: 1. Do not use waxed sheet paper or waxed containers where the time period is short or where unwaxed paper may otherwise be used. 2. Ask for and test out any available alternatives or substitutes, both to relieve the demand and to prepare for the possible necessity for substitution. 3. Use a lighter waxing where it can be satisfactorily used. 4. Where a thick coating of wax is used merely for its decorative merchandising value—sacrifice this for "the duration." This has already been done in the case of cellophane which is permitted as a wrapper in contact with a food, but cannot be used as an outer decorative wrapper for the same food.

C.J.B.

**351. Stokers.** ANONYMOUS. *Milk Dealer*, 31, No. 4: 24-25, 42. Jan., 1942.

A discussion is given of how stokers offer a solution to many plant problems. It is pointed out that evidence collected from users indicates beyond a doubt that boiler capacity can be stepped up considerably when a stoker replaces hand firing. In addition many other problems are eliminated, such as smoke nuisance, high fuel costs, shortage of manpower, and unsanitary boiler rooms that are a source of plant contamination. C.J.B.

**352. Large-Scale Organization in the Dairy Industry.** R. K. FROKER, A. W. COLEBANE, AND A. C. HOFFMAN, U. S. Dept. Agr., Cir. 527. 1939.

Since the close of the first World War there has been a marked development of large-scale corporations in all branches of dairy manufactures. Though there are several producer marketing cooperatives operating on a national scale, in general large-scale developments under the cooperative form of business enterprise have not been so rapid as those under the corporate form. During the period of 1925 to 1930 the total sales of all dairy products increased 12% while the total sales of the four leading dairy companies (National Dairy Products Corp., Beatrice Creamery Co., The Borden Co., and the Fairmont Creamery Co.) increased nearly 300%. In 1934 the largest dairy corporation handled milk products equivalent to 9.4% of the total volume of milk moving into commercial channels. (Growth of the large companies has been accomplished mainly through the purchase and consolidation of many small plants.

Meat packers and grocery chains are important handlers of dairy products. Many of the latter now own or control their own assembling and processing plants.

The cooperatives have shown little or no expansion in their scale of operations since 1930.

Capital investment in the four leading dairy companies earned from 16 to 18% in the six-year period 1925-30, 4.9% in 1932 and 9.2% in 1937. Profit margins ranged from as high as 7.3% in 1928 to as low as 3.2% in 1934.

The large companies control a larger percentage of the evaporated milk industry than they do butter and cheese. Concentration of control in distribution is greater than in the field of manufacture. Eleven dairy companies control about 18% of the total volume of fluid milk consumed in villages and cities in the United States. Three companies alone distribute 16%. In large cities the three largest dairies often control 60-90% of the total volume of market milk sold.

The use of the motor-truck in assembling milk from farms, better utiliza-

tion of by-products, and increased efficiency have been important factors in the growth of large dairy companies.

The distribution of butter shows less centralization than any other product. About 50% of the evaporated milk is made by three companies: Carnation, Pet, and Whitehouse (A & P).

The growth of mass distribution has resulted in a decrease in the importance of the middleman (the commission merchant, and broker, and the produce jobber).

Large-scale organization has not been extended to dairy-farming operations to any considerable degree.

There has been an increase in the unionization of labor employed in the manufacture and distribution of dairy products. This change has occurred largely in the large plants. Since unionization of labor usually means higher labor cost this factor may retard further consolidation and integration in the industry.

Patent rights constitute important instruments of economic control in the dairy industry. Outstanding examples have been the basic patents on process cheese and commercial uses of casein.

The part that cooperative marketing can play in a system of mass distribution is questionable but it seems that manufacturing and local assembling of the product are clearly within their province. The most satisfactory arrangement between the cooperative and the mass distributor is one under which their functional set-ups complement rather than duplicate each other.

P.H.T.

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## ABSTRACTS OF LITERATURE

### ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

- 353. A Comparison of Rats Fed an Evaporated Milk with Those Fed a "Milk" in Which the Naturally Occurring Fat Has Been Replaced by Coconut Oil.** SMITH FREEMAN AND A. C. IVY, Dept. Physiology and Pharmacology, Northwestern Univ. Med. School, Chicago.

The growth of rats fed an evaporated milk was greater over a 97-day period than for a similar group of rats maintained on a "filled milk" in which the butter fat had been replaced by coconut oil. The percentage of bone ash and of liver fat is quite similar for the two groups of rats, both after 49 and 97 days on the diets. There were more volatile fatty acids deposited in the storage fat of the coconut oil group.

- 354. A Colorimetric Method for Estimating the Quality of Butter.** G. KNAYSI AND E. S. GUTHRIE, Cornell Univ., Ithaca, N. Y.

A simple method of estimating the quality of butter is described. It consists in dissolving 1 ml. of the melted milk fat in 3 ml. of chemically pure xylol saturated with the free base of neutral red, and comparing the color with standards containing known quantities of oleic acid. The base of neutral red is prepared by precipitation with sodium hydroxide from an aqueous solution of the dye salt.

The test, which is a measure of the degree of hydrolysis of the butter fat, is found to be of value in quickly detecting bad samples and the majority of fair or good samples of butter, and in adding precision to the judgment of the expert.

- 355. A New Colorimetric Method for the Determination of Free Fatty Acids in Milk.** V. N. KRUKOVSKY AND G. KNAYSI, Cornell Univ., Ithaca, N. Y.

A simple and quick colorimetric method for the determination of free fatty acids in milk fat is described. The method is shown to be highly sensitive and accurate and consist in dissolving one ml. of the milk fat in 3 ml. of a saturated solution of the free base of neutral red in chemically pure xylol and comparing with a set of standards of known oleic acid contents. In neutral fat and in xylol, the dye base gives an orange yellow solution. Free fatty acids form red soaps with the dye base and the degree of shift to the red is proportional to the concentration of the soap and, therefore, of the free fatty acids. Equal normal concentrations of various fatty acids produce equal shift in the color.



**356. Further Studies on the Use of Basic Dyes for Measuring the Hydrolysis of Fat.** G. KNAYSİ, Cornell Univ., Ithaca, N. Y.

Four basic dyes, namely methylene blue, Nile blue, spirit blue and neutral red, are studied from the point of view of suitability in the study of fat. Preparation of the free bases from solutions of the dye salts is described, and the solubilities and stability of those bases under different conditions are studied. It is concluded that Nile blue is unsuitable on account of the instability of its base, and its use may lead to serious errors. The base of neutral red is found to be the most suitable on account of its stability and the ease with which it is prepared. In microscopic work, where excessive contrast is desirable, it is recommended that methylene blue or spirit blue be used.

**357. The Evaluations of Flavor Defects of Butter, Cheese, Milk, and Ice Cream as Designated by Dairy Products Judges.** G. M. TROUT, P. A. DOWNS, M. J. MACK, E. L. FOUTS, AND C. J. BABCOCK, Committee on Judging Dairy Products, A.D.S.A.

Evaluations of flavor scores of butter, milk, cheese, and ice cream by forty-seven trained judges and by a panel of five selected judges for each product were made. The judgments of the selected judges were found, for the most part, to be within a narrower range than those of the group of judges as a whole.

A knowledge of the classes or groups of off-flavors of butter, cheese, milk, and ice cream as arranged from the numerical scores given by the selected judges would seem to be of material value, 1, to those interested in becoming proficient in dairy products judging; 2, in unifying and standardizing dairy products judging throughout the United States; and 3, in furnishing a common basis for recording and evaluating research in which flavor data are involved.

**358. Ortho Phosphoric Acid as a Cheese Solvent.** V. CONQUEST, A. W. TURNER AND B. ROGERS, Armour and Company, Chemical Res. Lab., Chicago, Ill.

A method for the use of 1.0% ortho phosphoric acid as a solvent for cheese in the cheese sediment test is described. This method has the advantages of simplicity, low cost, and rapidity of execution, a combination not found in other similar techniques.

**359. Shark Liver Oil and the Vitamin A Potency of Milk.** L. L. RUSOFF, H. E. SKIPPER, AND P. T. DIX ARNOLD, Florida Agr. Expt. Sta., Gainesville.

Ten Jersey cows from the Florida station herd were placed in 4 groups receiving 0, 2.5, 5 and 10 pounds of shark liver oil (9,000 International Units

of vitamin A per gram) per ton of the mixed concentrates in the ration. The cows were subjected to three distinct feeding periods: pre-period before the use of shark oil, a winter or dry lot period, and a spring period on pasture. During the winter period all cows received clover hay.

Milk samples, taken at the end of each period, were assayed for vitamin A potency by slight modification of the U.S.P. XI biological technique. The vitamin A potency of the milk ranged from 1570 to 2035 I.U. per quart.

The addition of 2.5 pounds of shark liver oil per ton of feed (0.125 per cent of the concentrates) increased the vitamin A potency of the basal ration from 744,000 I.U. to 795,000 I.U. per cow daily, which was sufficient to produce milk of maximum vitamin A potency. Addition of shark liver oil above this level did not further increase the vitamin A potency of the milk.

Even when the cows were allowed pasture in addition, in the spring, no increase in the vitamin A potency of the milk was observed. One of these cows received orally an additional 1,400,000 I.U. of vitamin A daily in the form of shark liver oil (6 ounces) without further effect.

There was a slight tendency to depress the percentage of butterfat in the milk without affecting milk production.

The high vitamin A intake from the ration did not influence the ascorbic acid content of the milk.

## BACTERIOLOGY

- 360. Preserving and Germicidal Action of Various Sugars and Organic Acids on Yeasts and Bacteria.** F. J. ERICKSON AND F. W. FABIAN, Mich. State Col., East Lansing, Mich. Food Res., 7, No. 1: 68. Jan.-Feb., 1942.

The preserving and germicidal action of the sugars, fructose, dextrose, sucrose and lactose and the organic acids, acetic, lactic and citric, were determined, using thermophilic and mesophilic bacteria, several yeasts and a torula.

The order of activity for sugars was found to fructose > dextrose > sucrose > lactose on bacteria, the thermophiles being more susceptible than the mesophiles. The yeasts were more resistant than the bacteria to all the sugars, sucrose requiring 15% greater concentration than the hexose sugars for preserving indications, while lactose had no preserving action on yeasts. Fructose and dextrose, only, exhibited germicidal action on all yeasts. Sucrose was germicidal only to *Saccharomyces cerevisiae*.

The same method used for evaluating the preserving and germicidal activity of the acids indicated that on a percentage basis the effectiveness was lactic > acetic > citric for bacteria, whereas, on a pH basis, it was acetic > citric > lactic and that the mesophilic streptococci were more resistant than the thermophiles. These findings indicate that acids do not

depend entirely on the hydrogen ion for their action but partly on the anion and the un-ionized molecule. Yeasts were more tolerant of acids than the bacteria since a strength of between 0.5 and 5 normal were necessary to show activity which was had with concentrations of between 0.1 and 0.3 normal on the bacteria.

For bacteria the order of effectiveness of the acids in combination with the sugars was lactic > acetic > citric and again fructose and dextrose were much more active than sucrose. No germicidal action was exerted by lactose in combination with any of the preserving quantities of the acids.

In an acid and sugar combination the acid is the more important factor of the two in producing a germicidal effect on microorganisms.      F.J.D.

- 361. A Group of Coliform Bacilli Serologically Related to the Genus *Salmonella*.** C. A. PELUFFO, P. R. EDWARDS, AND D. W. BRUNER, Ky. Agr. Expt. Sta., Lexington, Ky. Jour. Infect. Dis., 70, No. 2: 185-192. Mar.-Apr., 1942.

A group of 7 paracolon strains, all of which liquefied gelatin and fermented lactose slowly, were compared serologically with *Salmonella düsseldorf* and *S. cerro*. The paracolon organisms displayed only slight relationships to the known *Salmonella* types, but the H antigens were closely related to those of *S. düsseldorf* and *S. cerro*. The 7 strains represented 5 serological types, the antigenic compositions of which were studied. The biochemical characteristics of these 7 strains differ from those of any of the genera now recognized.      J.F.C.

- 362. The Brucella Complement Fixation Reaction.** BOWMAN WISE AND H. W. CRAIG, Duke Univ. School Med., Durham, N. C. Jour. Infect. Dis., 70, No. 2: 147-151. Mar.-Apr., 1942.

The Brucella complement fixation reaction was found to possess no advantage over the more easily performed agglutination tests, except that the complement-fixing antibodies frequently appear before agglutinins in acute Brucellosis.      J.F.C.

- 363. The Nutritive Requirements of the Salmonellas. II. The Typhoid Bacillus: Carbon Source and Amino Acid Requirements.** WILLIAM BURROWS, Univ. Chicago Dept. Bact. and Parasitol. Jour. Infect. Dis., 70, No. 2: 126-130. Mar.-Apr., 1942.

In a basal medium of inorganic salts six carbon sources were used in testing the ability of 11 strains of typhoid bacilli to use various amino acids. The results on the utilization of amino acids varied not only with the different strains of organisms but also within strains as the carbon sources were varied.      J.F.C.

## BUTTER

- 364. Recent Russian Food Research.** ANONYMOUS. *Food Mfg.*, 17, No. 3: 72-73. 1942.

Methods are described for the use of a colloid mill in the production of synthetic foods of the "butter" type. It was found that under certain conditions butter-like products can be prepared from emulsions composed of milk, fat and water; this butter is said to have a better taste and constitution than ordinary margarine. It is also stated that production is very economical.

J.C.M.

- 365. The Flavor of Butter.** ANONYMOUS. *Food Mfg.*, 17, No. 2: 40-41. 1942.

This article goes into the history of butter making with emphasis on discoveries which led up to present-day ways of controlling the flavor by means of a starter.

J.C.M.

- 366. Butter in National Health.** CHRIS L. CHRISTENSEN, Univ. Wis., Madison, Wisconsin. *Med. Jour.*, 41, No. 1: 48. Jan., 1942.

Butter is a concentrated food which furnishes large amounts of energy; it is an excellent source of certain vitamins and accessory food substances which promote growth, health and well-being. Butter ranks second only to fish-liver oils in ease of digestion and completeness of utilization. Vitamin A and carotenes are present in good butter in varying proportions. About two ounces per day of good butter will supply the vitamin A requirements of an average child, while two to three ounces plus a pint of milk will be adequate for an adult's vitamin A needs. All butter is a fair source of vitamin D. A new unnamed growth factor also present in butter is being investigated.

W.V.P.

- 367. Use of Cereal Anti-oxidant to Prevent Tallowiness in Butter.** W. J. CORBETT AND P. H. TRACY, Univ. Ill., Urbana, AND C. N. HANSEN, Beatrice Creamery, Champaign, Ill. *Natl. Butter and Cheese Jour.*, 33, No. 4: 16. April, 1942.

See *JOUR. DAIRY SCI.*, 25, No. 1: A9. Abstract 21.

W.V.P.

## CHEESE

- 368. Right Way to Make Processed Cheese.** C. R. BARKER. *Food Indus.*, 13, No. 12: 53-55. 1941.

This article presents a technological method for preparing processed cheese. Making processed cheese is a science, not an art. Familiarity with the art only helps to get the desired results.

Mr. Barker outlines in this article what he considers the best procedure for making processed cheese. J.C.M.

- 369. Smoked Cheese.** J. C. MARQUARDT, N. Y. Agr. Expt. Sta., Geneva, N. Y. Natl. Butter and Cheese Jour., 33, No. 4: 20. April, 1942.  
(Also published in Vol. 8, No. 1, Farm Research, Geneva, N. Y.)

Smoking cheese adds flavor; wood smoke, "liquid" smoke or smoked salt may be used. When wood smoke is used its temperature should be kept below 100° F. and exposure should be from 18 to 36 hours. Three to 5 pound loaves should be cut lengthwise into two equal parts and smoked on wire grids. "Liquid" smoke may be added to the milk or to the cheese if it is to be processed; legal requirements are established for inter-state trade. Smoked salt introduces a "streaking" problem and lacks sufficient flavor. W.V.P.

- 370. Suggestions on Complying with Federal Cheese Standards.** A. T. BRUHN, Wis. Dept. Agr., Madison. Natl. Butter and Cheese Jour., 33, No. 4: 42. April, 1942.

The qualities of acceptable cheese are described. Such cheese is made from good milk which is protected and not unduly agitated in transit to the factory. Methylene blue and sediment tests are necessary to check production methods. Salt must be well dissolved and absorbed when curd is put to press. W.V.P.

- 371. How to Use Waste Rind in Processed Cheese.** SIMON BRICKNER. Food Indus., 14, No. 3: 47. 1941.

The thick, tough rind of natural Swiss cheese can be used in the making of processed cheese. The rind, which contains approximately 35% fat and 20% moisture, can be reduced to a fine powder by means of a special hammer mill.

It has been found by experience that up to one third of the batch may be replaced by this powdered rind without impairing the flavor, body, texture, or slicing qualities. J.C.M.

- 372. Utilization of Swiss Cheese Rind in Process Cheese.** SIMON BRICKNER, Dairy Chemist, Brooklyn, N. Y. Natl. Butter and Cheese Jour., 33, No. 3: 28. March, 1942.

See preceding abstract.

W.V.P.

## CHEMISTRY

- 373. The Pennsylvania Method for Determining the Percentage of Fat in Dairy Products.** W. D. SWOPE. Pa. Agr. Expt. Sta. Bul. 412.

A method was developed primarily for determining the fat percentage

in dairy products to which sugar has been added. Twenty-five reagents in varying amounts and combinations were tried. It was found that 2 milliliters of ammonium hydroxide (28 to 29%  $\text{NH}_3$ ), 3 milliliters of butyl alcohol (B. P.  $117^\circ \text{C.}$ ) and 17.5 milliliters of sulphuric acid (sp. gr. 1.72 to 1.74) gave results which checked favorably with the Mojonnier Method. Of 529 fat determinations made by the Pennsylvania Method on ice cream, ice cream mix, sweetened condensed milk, and chocolate milk, 84.3% were within  $\pm 0.19\%$  of the Mojonnier method. Author's Abstract.

- 374. Vitamin Methods. I. An Improved Procedure for Estimating Vitamin B<sub>1</sub> in Foodstuffs and Biological Materials by the Thiochrome Test Including Comparisons with Biological Assays.** LESLIE J. HARRIS AND Y. L. WANG, Dunn Nutritional Lab., Cambridge Univ. and Med. Res. Council. *Biochem. Jour.*, 35, No. 9: 1050. 1941.

An accurate procedure, based on the method of Wang and Harris (1939) for urine, has been worked out. This method has been tested with dairy products as well as numerous other foodstuffs.

Special features include: (a) A preliminary process of extraction, (b) digestion with papain and takadiastase, (c) washing of the digest with isobutanol, (d) omission of adsorption, (e) conversion into thiochrome in presence of methanol, and with addition of the  $\text{K}_4\text{Fe}(\text{CN})_6$  before the  $\text{NaOH}$ , (f) washing of the thiochrome layer with water, (g) visual comparison of fluorescence with the aid of light filters and blank controls. V.C.S.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

- 375. Preventing Off-flavors in Dried Whole Milk.** E. L. JACK AND J. L. HENDERSON, Davis, California. *Food Indus.*, 14, No. 3: 50-51. 1942.

Although dried whole milk represents the greatest degree of concentration of any milk solids product, its production is much less than other less highly concentrated milk products. The main reason is that up until recently it could not be produced with satisfactory keeping qualities.

It was found that oxidation caused the off flavor, and that it could be corrected for a period of two years by using the "atmospheric roller dryer" process when the milk was preheated to  $175^\circ \text{F.}$  for 15 minutes. J.C.M.

- 376. From Cow to Cloth.** ANONYMOUS. *Food Indus.*, 13, No. 12: 47. 1941.

An important new by-product of the dairy industry is "Aralac," a translucent cloth made from casein which is extracted from skim milk. This fiber can be blended with wool, mohair, cotton, rayon, or fur in varying proportions.

This development is significant to the food industries in that it provides a new outlet for skim milk that may reach important proportions. J.C.M.

## DISEASE

377. **Studies on Brucellosis in Mexico. Comparative Study of Various Diagnostic Tests and Classification of the Isolated Bacteria.** M. RUIZ CASTANEDA, RAUL TOVAR, AND RAFAEL VELEZ, Dept. Med. Res., General Hospital and Public Health Service of Mexico, Mexico City. Jour. Infect. Dis., 70, No. 2: 97-102. Mar.-Apr., 1942.

Over a 4-year period 200 human cases of brucellosis were studied. Clinical diagnoses were supplemented with blood cultures (both with increased CO<sub>2</sub> tension and in ordinary atmospheres), agglutination tests, intradermal tests, and opsonic tests. Of the 200 cases, 93% gave positive agglutination reactions, 84% positive blood cultures, 80% positive intradermal tests, and 60% positive opsonic tests. The classification of 150 strains of the organisms showed that 143 were *Brucella melitensis*, 5 *Br. abortus*, and 2 *Br. suis*. The high prevalence of *Br. melitensis* infections is accounted for by the considerable use of goat's milk and goat's milk products in Mexico. J.F.C.

378. **A Study of Hemolytic Streptococci from a Horse Treated with Sulfanilamide after Streptococcal Bacteremia Developed during Immunization.** JESSIE L. HENDRY, N. Y. State Dept. Health, Albany. Jour. Infect. Dis., 70, No. 2: 112-118. Mar.-Apr., 1942.

Blood taken the day before death from a horse that had been under treatment for ten days with sulfanilamide yielded hemolytic streptococci which were more resistant *in vitro* to the action of sulfanilamide than was the standard culture with which the horse was originally inoculated. There was evidence that the resistance to sulfanilamide increased progressively during therapy. After 14 mouse passages there was no apparent decrease in resistance to the drug. Sulfanilamide-inhibiting activity was demonstrated in broth filtrates of both the standard strain and strains obtained from the blood and organs of the horse, but the inhibiting activity was produced sooner by the drug-resistant strains. J.F.C.

## FEEDS AND FEEDING

379. **Legume Silage in Dairy Feeding.** S. I. BECHDEL, R. W. STONE, P. S. WILLIAMS, AND F. R. MURDOCK. Pa. Agr. Expt. Sta. Bul. 411.

Results of four feeding trials with dairy cattle revealed that legume silage makes possible the feeding of smaller amounts of concentrates for milk production, and also concentrates with a relatively low percentage of protein. Alfalfa silage should be fed in connection with some hay and also

with corn silage if it is available. Alfalfa-molasses silage and alfalfa-phosphoric acid silage are equal in feeding value for milk production. Milking cows tend to go "off feed" when fed heavily on phosphoric-acid legume silage. The feeding of pulverized limestone with this silage is advised. Eighty pounds of molasses or 18 pounds of 73% phosphoric acid per ton of green alfalfa are considered the optimum amounts of preservative to use.

Author's abstract.

## FOOD VALUE OF DAIRY PRODUCTS

- 380. The Effect of Fortifying the Infant's Diet with a Cereal Enriched by Iron, Calcium, and Vitamin B<sub>1</sub>.** MAURICE L. BLATT, ELLIS HARRIS, HOWARD JACOBS, St. Vincent's Infant and Maternity Hosp., Chicago, Ill. Arch. Ped., 58, No. 2: 694. Nov., 1941.

The minimum daily requirements of the pre-school child are 0.75 grams of calcium, 0.75 grams of phosphorous, 7.5 milligrams of iron and 125 International units of thiamine. A quart of milk contains approximately 1.06 grams of calcium, 0.80 grams of phosphorous, 0.62 milligrams of iron and 143 International units of Vitamin B<sub>1</sub>. On the basis of feeding 2 ounces of milk per pound of body weight a quart per day is not consumed until about the sixth month when the infant weighs approximately 16 pounds. It is not until the sixth month, therefore, that enough milk is ingested to supply the daily need of phosphorous and Vitamin B<sub>1</sub> and a positive iron balance is never attained on milk alone. A farina cereal enriched by the addition of iron, calcium, phosphorus, and stabilized wheat germ was fed as a part of the dietary of a group of institutionalized infants and young children. A second group in the same institution concurrently was fed a mixture of three unfortified farinas purchased in the open market. A comparative study was made of the growth and development of the two groups of children and their tolerance for the cereals. The authors concluded that farina enriched with iron, calcium, phosphorous and wheat germ was well tolerated by normal infants, in the usual milk diet, did not increase the number of stools, nor did it cause constipation. The increase in weight and red blood count was superior to the group fed ordinary farinas. The height of the children fed the enriched farina showed a superiority over those fed control farinas, but the significance of this trend was not established.

J.J.S.

## MILK

- 381. Dairy Industry in the National Emergency.** C. E. WYLIE, Univ. Tenn., Knoxville, Tenn. Mimeograph Report No. 68. February 20, 1942.

This report reviews the situation in regard to the following materials



used in the dairy industry: burlap, tin, vanilla, sugar, cattle, molasses and rubber. It includes adjustments in marketing; milk requirements for the nation and for Tennessee. The goals of milk production are shown for each Tennessee county with suggested methods for increasing production. Factors favoring and handicapping this increased production are listed.

Author's abstract.

382. "Quick-time" Pasteurization of Milk. A. C. DAHLBERG, R. F. HOLLAND, AND R. K. MINER, Geneva, N. Y. N. Y. State Agr. Expt. Sta. Tech. Bul. 261. 1941.

It was shown in laboratory experiments published last year that certain advantages in the pasteurization of milk might be secured at temperatures higher than those now used. The present experiments were conducted under commercial conditions to try the new heat treatment of milk under controlled factory conditions.

The term "quick-time" pasteurization was used to designate the heat treatment given the milk. It consisted of a controlled time above 140° F. (60° C.) during which the milk was heated to the desired temperature and cooled again below 140°. There was no holding at the highest temperature attained.

Milk was successfully quick-time pasteurized at 177.5° to 169° F. (80.8° to 76.1° C.) with the time interval above 140° varying from 5 to 24 seconds. For example, milk was successfully pasteurized at 170° F. (76.6° C.) with 12 seconds total time to heat and to cool. At these higher temperatures the variation in the highest temperature attained could be greater than the present accepted pasteurization standards and still show proper pasteurization. There was a tendency toward a slightly better milk as produced by quick-time pasteurization. No data were secured with pathogenic bacteria.

Author's abstract.

383. The Nation's Milk Supply in War Time. NORMAN C. WRIGHT, Hannah Dairy Res. Inst., Kirkhill, Ayr. Milk Indus. 22, No. 1: 33. July, 1941. (Review of the original article published in full in Roy. Agr. Soc. Jour., England, Vol. 101, Part II, 1941.)

The author discusses some of England's wartime requirements for milk and milk products, the best methods of utilizing the available supplies of home-produced milk and methods by which such supplies can be maintained at an adequate level. During 1938 sales of milk and cream were remarkably constant, manufacture of milk products was seasonal, and over twice as much milk was used for butter as for the production of cheese or the manufacture of condensed and dried milk. For wartime use of liquid milk, expectant and nursing mothers, children under 5 years, and hospital patients are allowed 1 pint of milk daily. School children are allowed two-thirds of

a pint of milk daily while the remainder of the population is allowed one-third of a pint per head. Of the manufactured milk products, first priority has been given to condensed and dried whole milk, second priority is given to cheese, and third to butter. J.J.S.

**384. The Resazurin Test.** J. G. DAVIS AND D. W. WATSON, Natl. Inst. Res. in Dairying, England. Milk Indus. 22, No. 8: 37. Feb., 1942.

The authors suggest using an incubation temperature of 18° C. instead of 37° C. for the resazurin test for the following reasons: 1. It is easier to maintain a large sink of water 18° C. than at 37° C. over a period of several hours. 2. The resazurin test at 18° C. is on the average three times slower than at 37°. Since the recognized standard time for the resazurin test at 37° C. is one hour, a suitable time for the test at 18° C. is 3 hours. 3. A temperature of 18° C. is a fair criterion for the assessment of the keeping quality of milk which is usually maintained in the household at about this temperature. 4. Time lag errors which may be serious in short time (*e.g.*, one hour) tests can be ignored in a three hour test.

The writers believe that the temperature of 18° C. could be adopted as a standard for keeping quality tests, not only of milk but of milk products. J.J.S.

**385. Farm Milk Cooling Important in War-Time Program.** RICHARD MARKLEY, JR., Wilson Cabinet Co., Smyrna, Del. Refrig. Eng., 43, No. 3: 154. March, 1942.

The automatic maintenance of high water level on cans is indicated to be the efficient method for cooling and storing night's milking to be followed by cooling, or cooling and storing morning's milking. Diagrams illustrating how this is accomplished with the compartment and pump design of the wet storage tank accompany the article. It is emphasized that ample water to milk ratio is more efficient in utilizing compressor capacity than is the case where a small ratio is used and an ice bank on the evaporator is needed to hasten cooling of the milk. L.M.D.

## MISCELLANEOUS

**386. Canning and the Far Eastern Situation.** ANONYMOUS. Food Mfg., 17, No. 3: 57-59. 1942.

The entry of Japan into the war and the subsequent loss of Malayan tin are likely to have far-reaching effects on the British canning system.

This article brings forth just why tin is used in canning. It also discusses several means of cutting down on the tin used, in order to make the limited supply last longer. Methods such as using iron-lacquered cans for dry foods, glass jars for fruits, and a thinner tinplate are discussed.

J.C.M.

- 387. The Revolving Fund Method of Financing Co-operatives.** MARVIN A. SCHAARS, Univ. Wis., Madison. Natl. Butter and Cheese Jour., 33, No. 4: 9. April, 1942.

When co-operative organizations lose the patronage of their owners they cease to be co-operatives and lose exemption from income taxes and certain borrowing privileges. Under the revolving capital plan each patron must contribute to the capital structure a small sum per unit of product handled by the co-operative for him and for which he receives a certificate of equity in the organization. These retains are used to retire indebtedness, capital investments and certificates. When the association has acquired sufficient capital then certificates are redeemed in the order of issue. This plan tends to increase membership loyalty, interest and responsibility, and sounder business methods. W.V.P.

- 388. How to Make Your Equipment Last Longer.** PAUL H. MANDT, Assoc. Ed., Natl. Butter and Cheese Jour., Milwaukee, Wis. Natl. Butter and Cheese Jour., 33, No. 4: 12. April, 1942.

To conserve equipment: Keep it clean and dry; repair leaks, cracks or breaks immediately; keep electric motors dry; don't use steel wool for cleaning; avoid denting equipment; lubricate moving parts; don't throw things into unused equipment; keep equipment properly painted or varnished. W.V.P.

- 389. Notes on Methods of Chemical Treatment for Corrosion Control in Refrigerating Brines.** K. M. HOLADAY, Chemical Engineer, Anheuser-Busch, Inc., St. Louis, Mo. Ice and Refrig., 22, No. 4: 304. 1941.

Increasing the density of a brine solution decreases its corrosiveness. The greater the density of a brine solution the lower will be the solubility of oxygen in the solution. Since calcium chloride brines with a given freezing point have greater density than sodium chloride brines of the same freezing point, this may account for the greater corrosive properties of sodium chloride brines.

A corrosion committee of the A. S. R. E. has recommended the addition of approximately 540 p.p.m. of chromic acid to calcium chloride brines and 1080 p.p.m. to calcium-magnesium chloride or sodium chloride brines, and 30 p.p.m. to cooling waters. Caustic soda is recommended for adjusting the pH value after treatment so that the brines will be slightly alkaline, but pH values above 8.5 should be avoided in order to prevent damage to galvanized iron surfaces.

Some later recommendations call for as low as 20 p.p.m. of chromic acid, but such low concentrations are to be recommended only where dilution or loss of brine is impossible.

Treatment with chromic acid is favored over the use of sodium dichromate because the total cost is less, and it is more convenient for neutralizing small ammonia leaks. Graphs indicating corrosiveness of brines of varying densities, and of varying pH values are included. L.C.T.

**390. A New Type of Frozen Foods Locker.** F. W. KNOWLES, Northwest Baker Ice Machine Co., Inc., Seattle, Wash. *Refrig. Eng.*, 43, No. 3: 157. March, 1942.

The lockers described are like a subdivided filing cabinet accessible from both ends. Each locker unit, 16 ft. long, is divided into six 6-cu.-ft. lockers. These long locker units or drawers roll out on ball bearings, and as the locker unit does not roll out quite half way, it is overbalanced generally on the end within the cold space. Unfilled lockers at either end may have temporary weights placed in them if empty or lightly loaded with frozen foods to prevent over-balance when pulled out. Swing doors on the side admit the patron to his locker space. The ends of the locker units act as insulated plugs to the cold space. The principal advantage lies in the fact that the locker patron does not have to enter a low temperature room to gain access to his stored goods. L.M.D.

**391. Conservation of Freon.** F. H. FAUST, *Refrig. Eng.*, 43, No. 3: 149. March, 1942.

The minimum estimate for Freon in 1942 is set at 13,357,000 lb., with a "probable" estimate of 15,568,000 lb., calling for 21,800,000 lb. of carbon tetrachloride as the chief raw material. It is concluded that a Freon conservation program be instituted and that the raw materials needed be made available. The industry sub-committee suggested a program of conservation divided into three categories: Equipment Manufacturer, Sales Engineering and Specifications, and Installation Service. The points brought out in the latter should be musts for all users of Freon refrigerating equipment and are as follows:

- "1. Check all joints and points of possible leakage with 'halide torch.' It takes time, care and patience to find small leaks.
2. Do not recharge a system unless the leak has been found and repaired.
3. Weigh in exact charge of refrigerant specified and use the minimum charge required.
4. Discontinue practice of blowing out evaporators, lines, etc., with refrigerant. Use another medium such as dry air, nitrogen or carbon dioxide.
5. Exercise care in purging air from condenser; a lot of refrigerant can be saved in this procedure.
6. Exhaust and reclaim refrigerant from systems to be replaced or repaired.
7. Exhaust and reclaim refrigerant from supposedly empty refrigerant drums or service cylinders.
8. Pump down and lock refrigerant charge of all seasonal operation jobs in liquid receiver.

Carefully check system for leaks before releasing liquid and resuming opera-

tion at beginning of season. 9. Use minimum size dehydrator that is feasible for permanent dryer. 10. Return empty refrigerant cylinders to supplier as soon as possible. 11. Service cylinder fittings, valves and connections should be replaced when worn, or damaged. They should be protected when not in use. 12. Replace all leaky shaft seals and gaskets on compressors."

Comment: These would seem to be reasonable procedures even under conditions of normalcy. L.M.D.

**392. Scale and Corrosion Control in Water and Brine Systems.** J. A. HOLMES, National Aluminate Corp., Chicago. Ill. Refrig. Eng., 43, No. 3: 145. March, 1942.

In treating water for condensers, heat exchangers, etc., three possibilities may have to be met, these being scale, corrosion, and algae or slime. Molecular dehydrated poly-phosphates are recommended for scale prevention, especially where water is recirculated and cooled by a spray pond or tower, added in the make-up water at the rate of one to five parts per million. Over-concentration of the hardness in the system must be prevented to avoid precipitation. Organic materials also are used to prevent scale formation through their development of a coating over crystals which remain suspended in the water never growing large or strong enough to form scale. Combinations of organic materials and phosphates are effective in that the organic matter besides stabilizing the water also stabilizes the phosphates so that they remain in the molecular dehydrated condition.

The prevention of corrosion in recirculating cooling systems can be effected by the use of chromates, the use of alkaline materials, the use of the molecular dehydrated phosphates in heavy dosages. The use of an alkali with the phosphate is better if the water is very corrosive or acid.

When cooling towers and spray ponds are bothered with algae and other slime growths, treatment with chlorine, copper sulfate, and potassium permanganate may be followed, but the chlorophenate type of treatment has been very effective, using 1 lb. per 5,000 to 15,000 gal. of water two or three times per week. Alternate treatment with chlorophenate and copper sulfate is good where different types of algae are found.

Slime formation in condenser systems from the presence of iron bacteria may be controlled either by chlorophenates or chlorine, the latter being employed if the water is used for ice making.

Water used for air washing in air conditioning can be treated for slime prevention by chlorophenates at the rate of 500 p.p.m. Two dosages a week for light contamination, and three or four where heavy contamination is found are suggested. Such treatment does not prevent entirely bacterial and fungi infection of food materials and room walls from the conditioned air, but eliminates the air conditioning apparatus as a source. L.M.D.

**393. Instructions for Pumping Out Ammonia Systems in Case of Air Raids.** ANONYMOUS. *Refrig. Eng.*, 43, No. 3: 165. March, 1942.

In case of air raids, it is desired to confine the ammonia in the smallest space in order to offer the minimum size target to bomb hits. Receivers, condensers, and brine coolers fall into this category.

"1. When the air raid alarm is sounded, the king valve on the liquid line from the receiver shall be shut, cutting off distribution of liquid to the plant. 2. All available compressor capacity shall be started, in order to pump down the system as quickly as possible. 3. The engineer should make a complete round, closing all of the expansion valves except one, in order to minimize any back-flow in the case of a break in some part of the ammonia system. He should carry an ammonia mask ready for instant adjustment in position. Masks should be provided for all men remaining in the engine room. 4. Water hoses should be connected in the engine room ready to be played on any escaping ammonia. 5. In case of interruption of the condensing water supply, compressors shall be shut down immediately. 6. In case the power supply driving compressors or circulating pumps fail, everything shall be shut down. 7. If either 5 or 6 happens, close as many valves on the suction lines as possible, to isolate the ammonia into as many separate systems as possible, so as to minimize the hazard in case of a break in some part of the system. 8. When compressors are shut down, suction and discharge valves shall be closed.

After the 'all clear' is sounded, start system as usual. Keep damaged parts of system isolated until repairs can be effected."

L.M.D.



**I. A. R. I. 75.**

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